RESISTANCE IN MAIZE TO THE MAIZE STALK BORER, *BUSSEOLA FUSCA* (FULLER) (LEPIDOPTERA : NOCTUIDAE)

by

MICHAEL RONALD BARROW

Submitted in partial fulfilment of the requirements for the degree of

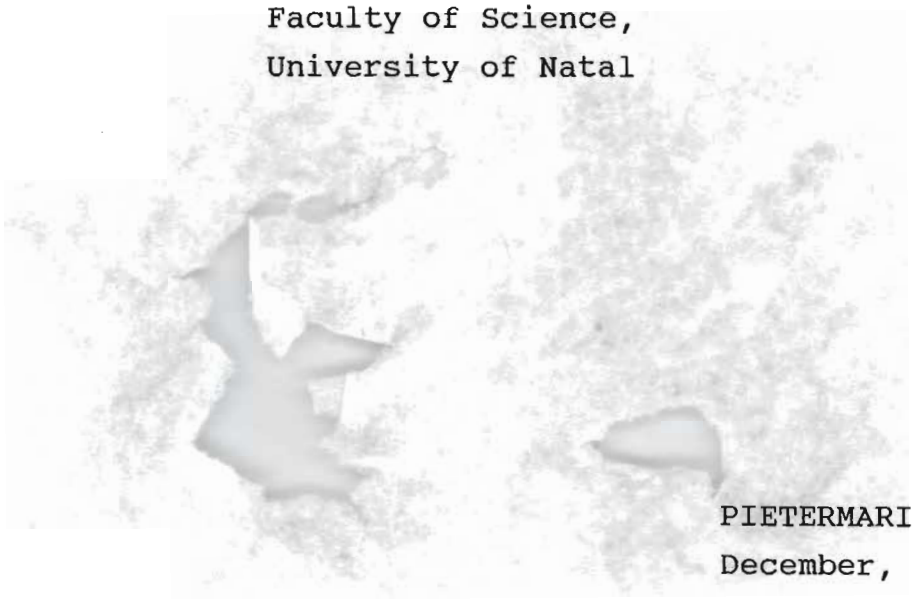
DOCTOR OF PHILOSOPHY

in the

Department of Zoology and Entomology

Faculty of Science,

University of Natal



PIETERMARITZBURG

December, 1989

"A new departure in the method of direct attack was made by Mr. A.M. Mostert in the spring of 1918. He tried crushing the young larvae by means of two little planks, each half an inch by 6 inches by 9 inches, with a suitable handhold, which he brought together smartly against the top of the plants a time or two" (Mally, 1920).

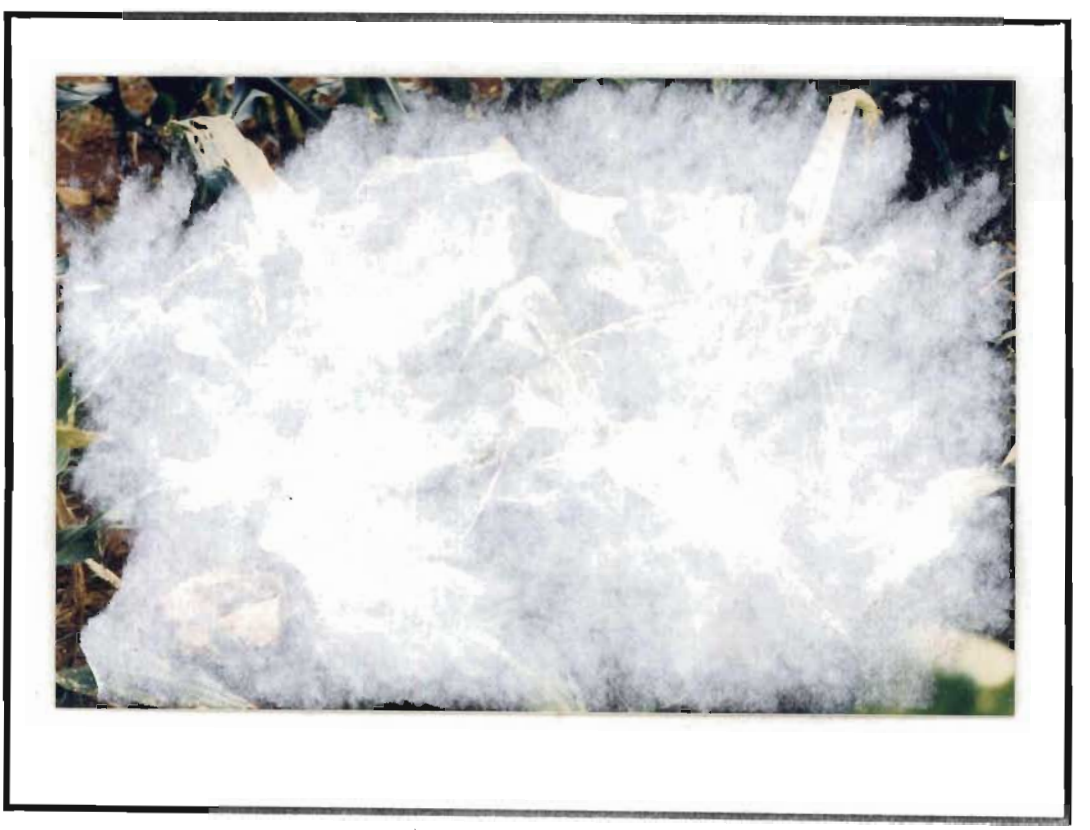


Plate 1. Maize plant severely damaged by *Busseola fusca*

P R E F A C E

- DECLARATION -

The experimental work described in this thesis was carried out at Pioneer Seed Company Research Farm "Hildesheim", Greytown, Natal, from the summer of 1981 to the winter of 1989, under the supervision of Professor Ted Bosman (1981 - 1987) and Professor Michael Samways (1987 - 1989).

This thesis is the result of the author's own original work, and contains no other work accepted for any other degree or diploma in any University, nor any material which has been previously published, except where due reference is made in the text of the thesis.

M.R. BARROW

ACKNOWLEDGEMENTS

The essence of research is to be able to follow one's hunches and intuition to finality, even if the conclusions are not of immediate benefit. I would like to express my appreciation to the Director of Research at Pioneer Seed Company, Ian Jarvie, for allowing me the independence to pursue those many avenues I deemed necessary in the search for resistance. The Board of Directors sponsored my registration, and I thank them most sincerely for their support.

I would like to state my appreciation, to Jackson Lekoko, Shobane Mokonyana and Zama Sithole, for many hours spent in routine laboratory and field work.

To many overseas researchers, mentioned in the text, who were most instructive in the early days of my research, I thank them for many hours of discussion and advice.

Professor Bosman was involved in the early planning stages of the project, and lent his ear for many discussions. I would like to thank him for his counsel and direction.

To Professor Samways I owe thanks for the motivation in finally writing up the thesis, and for constructive criticism on style and layout.

Messrs Derrick Charlton and Harvey Dicks, senior lecturers in the Department of Biometry, Natal University were intimately involved in the analysis of data. They were most helpful in organising the punching of data, and in discussing the interpretation of the results. I owe them a great deal of gratitude.

I would like to thank Cathy Rutherford-Smith most sincerely for her cheerfulness and exceptional accuracy in the typing of the manuscript. Annette Williams and Peter Cope also assisted greatly at the final stages and Anne Skelton and Andre Strydom assisted in the layout of several flow charts. I would like to thank them for their help.

ABSTRACT

An in depth review is given of Host Plant Resistance research on borers in general, and on *B. fusca* in particular. The general biology and economic importance of *B. fusca* are also discussed.

Several aspects of the general methodology of breeding for resistance to *B. fusca* are discussed. These topics include collection of overwintering larvae, termination of diapause, field infestation methodology and damage assessment. Approximately 20 first instar larvae were applied to the plant whorl when plants were about 30 - 40 cm tall. The primary method of damage assessment was to evaluate leaf damage on a 1 to 5 scale. Further criteria for selection of resistant germplasm are assessment of stunting due to stem boring and yield at harvest.

The effect of plant resistance on *B.fusca* was investigated. There were clearly defined differences in leaf damage evident between different maize genotypes. Heritability of this resistance was demonstrated, and presumed to be an additive mechanism that reduced insect feeding, indicating antibiosis. There were significant differences between the number of larvae recovered from whorl tissue of different cultivars. This was ascribed to two resistance mechanisms exerting their effects within the first few days' feeding by larvae. One mechanism was short lived, but effective, antibiosis resulting in larval death, while the other, also short lived, was repellence, resulting in larval migration. Both mechanisms resulted in fewer larvae feeding in the plants. Another longer lasting resistant mechanism affected larval growth and mass gain, resulting in smaller larvae. These mechanisms were found to be heritable traits.

Differences in levels of resistance affecting larval mass gain were also determined for various parts of the tassel. For all inbreds, the peduncles were more susceptible than the tassel stems and glumes.

Cultivars also differed in the levels of resistance in the stem tissue. Resistance in leaf tissue did not necessarily mean that resistance occurred in the stem of that genotype. Some cultivars had resistance mechanisms present in both leaves and stems, some had only one resistance mechanism in either part, and some were totally susceptible.

The effect of the borer on the plant was investigated. Leaf damage was found to not be of any consequence, but severe stem damage caused extensive yield losses. There was good correlation between leaf damage and stem damage. Yield loss was most pronounced in longer season hybrids than in quick maturing hybrids.

Methodologies utilized in the development of inbreds, populations and hybrids are discussed. It was concluded that borer resistant hybrids do have a place in the commercial market. However their performance under conditions of low or no infestation must be similar to that of other susceptible hybrids because control measures for *B. fusca* are not excessively expensive.

LIST OF CONTENTS

LIST OF FIGURES

LIST OF PLATES

LIST OF TABLES

CHAPTER 1

INTRODUCTION

1.1 DEVELOPMENT OF RESISTANCE TO PHYTOPHAGOUS INSECTS

The diversity of entomophagous insects is enormous, with at least a third of a million species feeding on the living tissue of higher plants (Strong *et al.*, 1984). A clash between phytophagous insects and man was therefore inevitable from the time that man grew crops for his own use.

Beck and Schoonhoven (1980) categorised phytophagous insects according to their host range. Monophagous insects feed on single plant species, or on several closely related species. Oligophagous insects feed on related families, and polyphagous insects feed on plants from more than one plant order. In addition to different plant species being consumed by one insect species, different feeding sites on the same plant at any one time are often consumed by one insect stage. Also, different stages of the same insect have different dietary requirements, and may utilize the same or different plant parts. Different growth stages of the plant can also be utilized by the same or different insect stages.

From the time that man first started improving his crop plants selection for better plants was practiced either deliberately or inadvertently. Man and insects have always competed for food and fibre, but it was only during the 20th Century that insect resistance was deliberately bred into plants. The expression of resistance in plants to insects ranges from slightly susceptible to totally immune. This is expressed as either a response by the insect to the plant, or results in an effect of the plant on the insect. And it is this natural variation in plant response that has led to the deliberate incorporation of resistance mechanisms in commercialised crops. Today millions of hectares of insect resistant cultivars are grown around the world (Gallun and Khush,

1980). This introductory chapter deals briefly with the terminology, development and usage of resistance in plants to phytophagous insects.

1.1.1 Types and classification of resistance

Resistance is the ability of a host plant to reduce, or withstand without adverse effect, insect damage. Horber (1980) stated that "Classification of resistance phenomena may express the relative success or failure of an insect species to survive, develop and reproduce on a plant species". Painter (1951) described resistance in plants as "the relative amount of its heritable qualities that influence the ultimate degree of damage done by the insect". Painter's system of classified degrees of decreasing resistance has generally been accepted and utilized by researchers in host plant resistance (HPR):

(i) **Immunity** - This is shown by a plant species that will not be damaged by a specific insect under any condition.

(ii) **High resistance** - This is illustrated by a small amount of damage caused to the plant by a specific insect under a given set of conditions.

(iii) **Susceptibility** - This is a plant reaction that shows more than average damage by an insect for the crop.

(iv) **High susceptibility** - A plant that shows much more than average damage (especially death) is considered highly susceptible.

Painter also mentioned certain other phenomena related to resistance, but which were not necessarily heritable traits.

(i)**Host evasion** - Occasionally normally susceptible plants may not be attacked by an insect. This can happen if the host plant passes through the susceptible stage quickly, or when the insect occurs in low numbers. Early maturity may also enable some cultivars to not be damaged. Artificial infestation will usually determine whether the lack of damage observed in the field is true resistance or evasion due to whatever factors.

(ii) **Induced resistance** - It is possible for a plant to sometimes show temporally increased resistance. This can result from some change in the condition of the plant or environment (soil moisture, ambient humidity, soil fertility). It is possible to utilize such induced resistance, but it is not a stable, nor a heritable, trait.

(iii) **Escape** - Occasionally a host plant may sustain no infestation due to some transitory circumstance such as incomplete infestation. An uninfested plant in a heavily infested population does not necessarily mean that it is resistant. Only studies of their progenies or artificial infestation will establish the true picture.

1.1.2 Biochemical and morphological types of resistance

Resistance in crops can range from temporal escape to the presence of lethal chemicals. Between these two extremes occurs a vast array of both chemical and morphological characteristics that can seriously disrupt the behavioral or metabolic processes of phytophagous insects. It is convenient to consider plant defenses under the major headings of biochemical and morphological bases.

(i) **Biochemical bases.** In the last 35 years knowledge of the chemistry of plants has increased substantially, and has enabled the biochemical bases of resistance to be determined.

The aglycone 2,4-dihydroxy-7-methoxy-2-1,4-benzoxazin-3-one (DIMBOA) was identified by Klun *et al.* (1966) in *Zea mays* as causing feeding inhibition to first instar larvae of *Ostrinia nubilalis* (European Corn Borer). This chemical resistance to *O. nubilalis* was an important discovery in HPR research that laid the foundations for future work on this and other lepidopterous species.

Other substances that resulted in plants being resistant to insects include Selenium, certain aromatic amino acids, and several secondary substances like alkaloids. Norris *et al.* (1980) gave an extensive list of chemicals that impart resistance in plants to insects. Present state of knowledge clearly shows that chemically based resistance is a major component of any plant's defence system against phytophagous insects.

(ii) **Morphological bases.** These are physical resistance factors that interfere physically with the mechanism of host selection, feeding, ingestion, digestion, mating and oviposition. They do not have any direct effect on chemically mediated behavioural and metabolic processes in the insect. Much of the existing man-enhanced resistance in crops has resulted from manipulation of morphological factors. The majority of recognized physical defence factors include thickening of cell walls, increased tissue toughness, proliferation of wound tissue, solidness and thickness of stem rinds, varying numbers, shape and stickiness of trichomes, accumulation of surface waxes, incorporation of silica and anatomical adaptations of non-specialized organs and protective structures.

The effects on insects are varied. These include interference with feeding and oviposition mechanisms, dehydration of eggs, digestion and oviposition, locomotion of the insect on the plant, lack of shelter and insect cuticle abrasion.

In conclusion, resistance to insects is rarely due to only one factor. This is especially true if several stages of the same insect utilize a host plant, or if different parts of the same plant are utilized by the same insect. By manipulation of several factors within a single host plant, man may be able to gradually gain the edge in the eternal competition with insects for food sources.

1.1.3 Mechanisms and inheritance of resistance

Painter (1951) proposed several mechanisms of resistance, which have been generally accepted by HPR researchers:

(i) **Non-preference** - When an insect avoids a plant for whatever reason, the rejection is taken as non-preference. These plants lack the characteristics that make them attractive as host plants, and are therefore not utilized as food sources, oviposition sites or for shelter. Kogan and Ortman (1978) proposed the term "antixenosis" to replace Painter's "non-preference". Both terms are used freely.

(ii) **Antibiosis** - This term covers all adverse effects exerted by plants on any aspect of an insect's biology. Typically these aspects include survival, development and reproduction.

(iii) **Tolerance** - Any plant that can tolerate an insect infestation without having its vigour or yield adversely affected would be termed tolerant to that pest. These plants show the ability to withstand an infestation that would normally result in severe damage to susceptible plants.

Obviously not all plant responses fit into these three categories, but they are very useful and workable definitions. These mechanisms may interact and compliment each other in the sense of intensifying expressions of resistance. It is quite possible for plants to have several mechanisms together, as will be described in this thesis. An interesting observation of evolutionary significance is that antibiosis and non-preference can exert selection pressure on pest populations, while tolerance does not.

Other useful terms, from a phytopathological view (van der Plank, 1968), are :

(i) **Vertical resistance** - This specific resistance is expressed against only some biotypes of a pest species. Generally only a single gene is present (monogenic resistance) or resistance is governed by a few genes (oligogenic resistance). The effects of monogenic resistance are quantitative. Segregation in the F_2 or later generations is clear cut and discreet. These genes are also referred to as vertical genes. Oligogenic resistance results in plants showing continuous variation from susceptibility to resistance in the F_2 or subsequent generations. The effects are thus qualitative, and each gene makes a small contribution to total resistance. Vertical resistance can, however be overcome by the formation of new insect or disease biotypes. For this reason, although occasionally of great use in resistance breeding, it is preferable to attempt to obtain polygenic resistance, which is a more stable type of resistance.

(ii) **Horizontal resistance** - This type of resistance (polygenic resistance) is effective against all biotypes of a pest species. As several genes are present in the plant, this type of resistance is more stable and longer lasting than vertical resistance. Multigenic resistance is sometimes used in the literature.

Occasionally, plants show a more resistant reaction in the mature stage than in the immature or seedling stages. This is termed adult plant resistance. The converse can also occur. Klun and Robertson (1969) demonstrated that maize plants in the whorl stage were more resistant to the European Corn Borer than plants in later stages of growth. This higher level of resistance was related to the higher level of DIMBOA present in whorl tissue than in plants at the tasselling stage. Reduced concentrations of DIMBOA in susceptible cultivars were correlated with decreased resistance.

Field resistance is a term used to describe resistance observed in the field which can often be different from resistance observed in laboratory or greenhouse.

Multiple resistance can also sometimes occur. This is the phenomenon that occurs when a cultivar is protected from different environmental hazards. These could include two or more of insects, diseases, heat, cold or pollution. The multiple resistance could be conditioned by the same gene or set of genes, or could be two separate mechanisms that give the plant resistance against two separate pests (Klun and Robertson, 1969). DIMBOA has been demonstrated to possess wide biological activity beyond that against European Corn Borer and is known to be a factor in resistance to stalk rot in maize (Bemiller *et al.*, 1965), to stem rust in wheat (Elnaghy and Link, 1962) and against damage to maize by triazine herbicides (Hamilton, 1964).

Nyhus *et al.* (1988) showed that the development of resistance to both European Corn Borer and *Diplodia maydis* was possible in two maize synthetics after four cycles of recurrent selection. They concluded that the genes governing resistance to both organisms acted in an additive manner. Selection for resistance to both organisms was associated with improvement in stalk rind strength coupled with decreases in the incidence of stalk lodging and natural stalk rot development. Currently, the author is assisting John Mihm [1] of CIMMYT in the development of multiple borer resistant maize germplasm. The other borers involved in the screening are Fall Armyworm (*Spodoptera frugiperda*), Sugarcane Borer (*Diatraea saccharalis*), and the Southwestern Corn Borer (*D. grandiosella*). Initial results (Mihm, pers. comm.) show that some of the resistant hybrids are highly resistant to the above 3 species. In addition, screening has recently been commenced against the European Corn Borer the Spotted Stem Borer (*Chilo partellus*), the Pink Stem Borer (*Sesamia calamistis*) and the African Sugarcane Borer (*Eldana saccharina*).

---

[1] Dr. J.A.Mihm, CIMMYT, Apdo. Postal 6-641,06600,Mexico,D.F.,Mexico

As with other agronomic traits, the type of insect resistance can be identified by the effects of variable gene expression (Gallun and Khush, 1980):

(i) Intra allelic

(a) **Recessive.** The F1 hybrids from resistant and susceptible parents are susceptible.

(b) **Dominant.** The F1 hybrids from resistant and susceptible parents are resistant.

(c) **Incompletely dominant.** The F1 hybrids from resistant and susceptible parents are intermediate in resistance.

(ii) Inter allelic

(a) **Complimentary.** Two or more genes together govern the expression of a trait. One of them alone is ineffective.

(b) **Additive.** Two non allelic genes affect the same character, and enhance each other's effect. One gene will be sufficient to show a useful level of resistance.

(c) **Epistatic.** One gene inhibits the expression of another gene.

1.1.4 Genetics of resistance to insects

The study of the inheritance of resistance to phytophagous insects has involved a fairly standard approach irrespective of the type of insect under study. Plants have been evaluated under insect pressure (natural or artificial infestation), and conclusions drawn on the response of either the insects to the plant or the plant to the insects.

Assessment of plant injury may range from a simple rating of visible leaf damage to a complex analysis of several factors including damage to several plant parts and yield losses. Insect response is normally measured as restlessness, weight loss, avoidance of the plant as a food or oviposition source, extension of the life cycle, reduced fecundity and oviposition, or death.

Generally, as it requires less labour, time and financial inputs, the reaction of the plant to the insect is evaluated. Initial selection of resistant material is based on a rating of damage on a simple scale. Thereafter, genetic analysis can be utilized to obtain an idea as to the inheritance of the resistance. Hybrid populations from the crosses of resistant and susceptible parents are evaluated. The reaction of the F1, F2 and sometimes the F3 and backcrosses are then assessed to determine whether the resistance is recessive, dominant or incompletely dominant, quantitative or qualitative.

There are several essential requirements that are obligatory in any HPR research:

(i) A genetically uniform population of the insect is required. The more uniform it is, the easier it is to identify differences in plant response as being due to the heterogeneity of the host plant population, and not to variation in the insect population.

(ii) Large numbers of healthy insects are required for infestations. This generally necessitates an efficient mass rearing technique.

(iii) In comparing cultivars for levels of resistance, homozygous plant material should be used. The resistant and susceptible checks, so essential in monitoring the severity of the infestation, must also be pure lines. Where one is searching for sources of resistance within plant populations, the more heterozygous the population the better the chance of identifying resistant and susceptible material.

(iv) Efficient response evaluation techniques are required for determining the plant/insect interactions. Frequently the evaluation of large volumes of segregating plant material is required. As previously mentioned, often a method which rates the plant response to the insect is the simplest.

The genetics of resistance has been investigated for at least 3 borers in maize: European Corn borer, Corn Earworm, and Fall Armyworm. A brief description of the findings follows:

European Corn Borer (*Ostrinia nubilalis*): Marston (1930) first showed that the resistance to *O. nubilalis* (in a variety Maize Amargo) was due to a single recessive gene. Schlosburg and Baker (1948) suggested that borer resistance was additive, due to the combined effects of several genes. Singh (1953), investigated resistance in the inbred A279 and concluded that 2 genes were involved. Penny and Dicke (1956) studied the resistance in 2 single cross hybrids and concluded that 3 genes were involved. It is evident that the maize genotype under study has a strong bearing on the conclusions regarding the genetics of resistance.

Corn Earworm (*Heliothis zea*): Several groups of researchers investigated the resistance to *H. zea* and all concluded that resistance is inherited quantitatively (Robertson and Walter, 1963; Widstrom and Hamm, 1969).

Fall Armyworm (*Spodoptera frugiperda*):
As with the Corn Earworm, the mode of inheritance was found to be quantitative (Widstrom *et al.*, 1972).

1.1.5 Use of resistance in pest management schemes

The field of HPR research is growing wider and involving more and more scientists. An informal newsletter edited by Foster and Ortman (1988) presents preliminary data from many researchers active in the field of HPR. Thirty crops are mentioned and 96 insect pests are under investigation. However, problems may arise in the exclusive use of resistant crops to control insects. High levels of resistance controlled by only one or two genes may lead to the development of new insect biotypes. Faris *et al.* (1976) showed that some sorghum cultivars that were initially resistant to Sorghum Midge soon showed a breakdown in resistance to the insect. The reports of breakdowns in resistance have been on multi-generation insects that have a short life cycle. This renewal of life stages over a short period of time predisposes the selection of new biotypes in response to any adverse environmental changes. No apparent breakdown in resistance to borers (which generally only have one to three generations per year) has been reported yet.

Painter (1951) stated that for resistant varieties to be effective, they must be integrated into control systems designed for specific pests and into the improvement programme of particular crops. Other pest control methods must also be considered and utilized fully. Ideally, resistant varieties should provide complete and permanent control of a pest. Such high levels of resistance have only been developed in a few crops to control a few pests. However lower levels of resistance are extremely useful if integrated with other control methods such as biological agents, changes in planting dates, early harvesting, crop sanitation, crop rotation and destruction of overwintering or alternate hosts. Even crops with low or moderate resistance offer several advantages in integrated pest management (IPM) systems. The reduction in pest numbers achieved through

resistant varieties is continuous, cumulative, low cost, and can make chemical or cultural control easier or cheaper (Pimentel, 1969; Dahms, 1972; Maxwell, 1972).

Painter categorized the use of resistant crop varieties as follows:

(i) **Principal control method.**

The literature abounds with references to the use of resistance as the principal means of control. These instances have mainly involved insects which have a high host specificity, such as aphids and scales (Painter, 1951). Control of *O. nubilalis* by insecticides and cultural control has generally not been satisfactory. Major research efforts were directed towards the development of borer-resistant maize (Brindley and Dicke 1963), and in one of the few economic estimates as to the extent of the use of resistant maize, Luginbill (1969) estimated the value of the resistance during the period 1962-1969 to have exceeded 150 million dollars annually. Major efforts have recently been directed toward the development of varieties resistant to the second generation of *O. nubilalis*.

Another borer for which resistant varieties are used as a principal control measure is *H. zea*.

(ii) **Varietal resistance used in conjunction with insecticide control.**

This is the most widely used form of integrated control, where the careful timing of insecticide applications occurs. Maxwell (1972) mentioned that toxic substances in the plant may make the pest more susceptible to certain insecticides or naturally occurring pathogens. By planting a resistant variety, a continuous level of suppression on each pest generation is maintained. This slows population growth and reduces the number of insects each generation. These factors can accumulate over seasons possibly reducing

pest populations to sub-economic damage levels. Insecticide applications may therefore not be required so frequently.

If the resistance is morphological, the effectiveness of insecticide applications may be enhanced, for example through a more open plant structure. This is a selection criterion for sorghum, where open panicles are preferred over tight panicles for control of *Heliothis* sp. Changes in plant morphology may allow the predators to find their prey more easily, leading to reduced pest populations.

In the above cases, the use of resistant plants may allow a reduction in the amount of insecticide and number of applications. The incorporation of a resistant variety into an IPM scheme is a better system of crop protection than complete reliance on insecticides to protect the susceptible variety (Pathak, 1975).

(iii) Varietal resistance used in conjunction with biological control.

As they do not greatly affect natural enemies of pests, resistant varieties are highly compatible with biological control systems. Low levels of resistance result in low pest populations remaining on the crop, and these serve as hosts for the natural predators, parasitoids or pathogens. The major advantage of using resistant plants in an IPM system is the preservation of the natural enemies. Pathak (1975) suggested that restless movement of pests on resistant varieties may expose them more to predator activity. Maxwell (1972) commented that resistant plants could reduce pest vigour, thus improving predator efficiency. Dahms (1972) stated that delayed insect development could result in immature stages being exposed to natural enemies for longer periods of time.

In conclusion, the examples discussed above illustrate chiefly

how resistant varieties can affect the population dynamics of pests, and how this disruption or change can be integrated with other control methods. Integrated control relies on maximizing all types of natural insect control methods while minimizing insecticide applications. Development of resistant plant varieties plays a key role in this holistic approach.

1.2 RESISTANCE IN MAIZE TO BORERS

Global maize production was estimated in 1986 (CIMMYT, 1987) to have been approximately 480 million metric tonnes. This makes it the second largest crop in the world after wheat (530 million metric tonnes). Maize shows great genetic diversity, and is therefore capable of being grown in many different environments. It occurs in areas from latitudes 50 degrees North to 42 degrees South and at elevations from sea level to 3800m above sea level. It is grown in lowland temperate, sub-tropical, and tropical areas, and in temperate, sub-tropical and tropical highlands (Ortega, 1987). In all these areas maize is attacked by various borers and leaf eaters which belong primarily to the two families Noctuidae and Pyralidae.

The most important borers and leaf feeders are: *Ostrinia nubilalis* (European Corn Borer) in the Northern Temperate regions, *O. furnacalis* (Oriental Corn Borer) in South East Asia, *Chilo partellus* (Spotted Sorghum Stem Borer) in East Africa, South Africa, Australia and South East Asia, *Busseola fusca* (African Maize Stalk Borer), *Sesamia calamistis* (African Pink Stem Borer), *Eldana saccharina* (African Sugarcane Borer), *Spodoptera exempta* (African Armyworm) all of which occur throughout Africa south of the Sahara, *Diatraea saccharalis* (Sugarcane Borer) in the broad area from the South Eastern U.S.A. to the Argentinean corn belt, *D. grandiosella* (Southwestern Corn Borer) in the U.S.A. and Mexico, and *D. lineolata* (Neotropical Corn Borer) in Eastern Mexico, Central America, and the Caribbean region. Tams and Bowden (1953) carried out a revision of borers on graminaceous crops in Africa. They listed 26 species from 6 genera of damaging borers, some of which are only minor pests. Others, like *B. fusca* and *S. calamistis* are major pests of grain crops. Harris (1962) also surveyed the Lepidopterous borers of cereals in Nigeria. He mentions 11 stem boring species of Lepidoptera feeding on 7 major cereal crops. In addition, 6 indigenous grasses were also infested. Ortega (1987) states that *B. fusca* is regarded as the most important pest of maize in sub-Sahara Africa at altitudes above 500m above sea level. In Africa

the noctuid borers predominate, whereas the pyralids are more common in the Americas, Asia and Europe. Damage by these insects can be caused to the leaves, ears, tassels and stems. Only a few species have been considered of enough significance to attempt to develop resistance in their host plants.

1.2.1 Historical review

Investigations into the development of resistance to borers began in the late 1920's on the European Corn Borer (*O. nubilalis*) (Dicke, 1954; Guthrie *et al.*, 1960). Roubaud (1928) artificially infested 5 French varieties of maize with newly hatched larvae and found almost complete mortality of larvae on a variety called Dent de Cheval, as did Hase in another study (Hase, 1929). In South Africa, Ellinger and Chorine (1930, 1931) found that *O. nubilalis* was similarly affected by a Natal variety which was thought to be of the same origin as Dent de Cheval. Other early research was carried out by Marston (1930, 1933) using a variety called Mays Amargo. This variety was also found to be resistant to attack by *O. nubilalis*. Marston was also the first to investigate the heritability of the resistance. He showed that the resistance in Mays Amargo was transmitted to the progeny of its crosses. Since those early days, investigators have discovered tremendous variability in levels of resistance in different maize populations, and HPR research has mushroomed in the past decade.

Early research work on *O. nubilalis* relied on both natural and artificial infestations. Variations in the attractiveness of different maize varieties were recognised very early on. Various researchers reported on the importance of height in the level of attractiveness of maize to moths (Marston and Dibble 1930; Ficht, 1936; Patch,1942). Artificial infestations were commenced in 1932 (Guthrie *et al.*, 1971). Infested stalks were removed from the field and placed in large cages. Moths were collected in Spring and eggs were used for artificial infestations. Small waxed paper discs containing egg masses were dropped into the plant whorl, and the damage to the leaves was evaluated. More recently,

laboratory cultures of many Lepidopterous larvae have enabled more precise and numerous field infestations to be carried out (Atkinson, 1978 on the Sugarcane Borer *Eldana saccharina*; Davis, 1980 on Southwestern Corn Borer; Mihm, 1983 on various maize stem borers; Schroeder *et al.*, 1986 on European Corn Borer). Recently, researchers at CIMMYT in Mexico developed a technique for infesting plants with larvae of several lepidopterous species (Ortega *et al.*, 1980). This eliminated laborious egg handling and reduced predator attacks on eggs. It involves the use of a manual applicator as described in section 3.2. below, and has been adopted by many researchers working on several pests (Wiseman *et al.*, 1980 on Fall Armyworm; Hall *et al.*, 1980 on Tobacco Budworm (*Heliothis virescens*); Davis *et al.*, 1980 on Southwestern Corn Borer).

The rating of damage has involved assessment of several damaged plant parts. The quickest and most widely accepted method of rating leaf damage was developed by Guthrie *et al.* (1960) for *O. nubilalis*. This is a 1 to 9 scale rating system based on the amount of leaf damage caused by larvae feeding in whorl tissue and is extensively used by HPR researchers. Additional measures of the level of resistance expressed by damaged plants include measurement of stem cavities caused by boring larvae (Umeozor *et al.*, 1985; Guthrie *et al.*, 1985; Jarvis *et al.*, 1986), counts of entrance holes (Ghidiu *et al.*, 1979; Lynch *et al.*, 1980;), yield losses (Jarvis *et al.*, 1986; Klenke *et al.*, 1986) larval counts (Grier and Davis, 1980; Guthrie *et al.*, 1982; Davis and Williams, 1986;) and stalk breakage (Davis and Williams, 1983; Jarvis *et al.*, 1986).

The identification and development of resistant germplasm has had from nil to considerable impact on the production of resistant commercial cultivars. As commercial companies do not disclose the pedigrees of their hybrids, it is not possible to ascertain the utilization of such resistant material in the market place. Overman (1986) discussed the major discoveries from the public sector and their impact on commercial seed research. He quoted research by Williams and Sanford (1983) which showed an estimated profit from Fall Armyworm resistant maize hybrids under borer attack, but not for commercial but susceptible hybrids. Overman concluded that Fall Armyworm resistant hybrids would have a significant marketing advantage over present susceptible hybrids, but gave no data as to their present commercial use. Communication with various researchers (Barry [2], Davis [3], Guthrie [4], Wiseman [5],) in HPR has elicited generally less-than-hopeful prognoses on the use of borer resistant maize germplasm. Barry (pers.comm.) wrote in 1985: "During the 1950's we were fortunate to have developed inbred lines with good combining ability for resistance to the first generation of the European Corn Borer. These lines were used very effectively through most of the 1970's. However, beginning in the late 1970's and into the 1980's, inbreds used in single cross hybrids were developed with greater yield potentials. The maize producers decided it was worth the risk to plant the higher yielding hybrids, and if a stem borer problem developed, they could treat and easily pay for the insecticide application". Hallauer [6] (pers. comm.) stated

---

[2] Dr. D.Barry, Department of Entomology, College of Agriculture, Columbia, Missouri, 65211

[3] Dr. F.M.Davis, Crop Science Research Lab., Corn HPR Research Unit, P.O. Box 5367, Mississippi State, MS 39762

[4] Dr. W.D.Guthrie, Corn Insects Research Unit, Box 45B, Ankeny, Iowa 50021

[5] Dr. B.R.Wiseman, Insect Biology & Population Management Research Lab., P.O. Box 748, Tifton, Georgia 31793

[6] Dr. A.R.Hallauer, Department of Agronomy, Iowa State University, Ames, Iowa, 50011

that cornborer resistance is not a major selling point of new hybrids. Overman (1989) however stated that DeKalb-Pfizer Genetics had developed hybrids with resistance to multiple species of leaf-feeding and stalk-boring Lepidoptera which were competitive with the best commercial hybrids.

The current status in maize HPR research is that investigations are ongoing, with a major emphasis on the development of resistant, but agronomically sound, inbreds for combination in resistant hybrids.

1.2.2 Requirements for a resistance programme to borers

There are several basic components of any HPR programme that need to be implemented if success is to be achieved. Mihm (1983) mentioned the following major areas:

(i) A colony of insects which is as vigorous and damaging as the endemic pest population.

(ii) An efficient mass rearing facility which will produce sufficient numbers of the particular insect stage required for artificial infestation.

(iii) Access to a wide range of germplasm that is representative of the genetic variation within the crop under study.

(iv) Methods for artificial infestation which will produce damage similar to that occurring under natural infestation.

(v) Methods for the assessment of the interaction between plant and insect.

(vi) An effective breeding programme to fully utilize and integrate the resistance into commercially acceptable cultivars.

A flow chart for a hypothetical programme for HPR development was presented by Mihm (1985) in a review on breeding for HPR to maize stem borers. These procedures are listed with comments below.

(i) Detection and identification of insects attacking plants.

(ii) Review of literature on the pest. It is very rare that no literature exists on a pest damaging an established crop. A comprehensive literature search is an essential pre-

comparison purposes. Various ratings must be utilized to differentiate levels of damage between cultivars. These ratings generally involve assessment of plant damage or, occasionally, the effect of the plant on the insect.

(vii) **Locate sources of resistance.** Extensive screening of diverse germplasm will hopefully result in detection of germplasm more resistant than the norm.

(viii) **Utilize resistance.** Once identified, the resistance must be developed into a usable form. In maize, this would be in the form of resistant inbreds or populations. Recognised plant breeding methods would be utilized to accumulate and increase the number of resistant genes from different sources.

(ix) **Measure effectiveness of resistance.** To be worthwhile, resistance in a variety to a particular insect must show a benefit in yield or any other important attribute over that produced by a susceptible variety. Generally this would be expressed as a reduction in yield loss when the variety was infested. The benefit could also be of a longer term nature, in that the pest population could be gradually reduced over seasons to levels that required no chemical usage.

(x) **Determine genetics of resistance.** Although an important aspect in HPR investigation, it is not essential to know the type of gene action or how many genes are involved in the resistance to a particular insect. These studies can be involved and lengthy, and successful development of resistant germplasm can occur with no knowledge of the genetics. It is soon apparent in any investigation whether there are one or more genes involved. The fewer the genes, the easier it is to develop resistance in elite material through a backcrossing programme, but lack of knowledge on the genetics is not a recipe for failure.

(xi) **Determine how resistance genes affect the insect.** Any resistant germplasm must obviously have an effect on one or several of the life stages of the invading pest. As with knowledge on the genetics of resistance, it is interesting

but not essential, to know how the insect is affected by the resistance. As long as the variety is resistant and a decrease in damage is accomplished, it is sufficient for successful exploitation of that resistance.

(xii) **Determine nature of resistance.** It is interesting, but not vital, to have an insight into the nature of resistance. It will have no bearing on the acceptance and utilization of the resistant germplasm whether the resistance is tolerance, non-preference or antibiosis. If the resistance works, use it!

The development of an HPR programme is a lengthy process. The basic principles (mass rearing, infestation techniques, screening, rating damage) are often fairly easy to develop and within a few years can be refined to provide a streamlined process. The actual development of resistant germplasm takes several years before any worthwhile material can be developed. This material must be agronomically acceptable if it is to be of any use in the development of commercial hybrids. From commencement of an HPR programme in maize to actual commercial sales of a resistant hybrid may take between 10-12 years. This time period is greatly dependent on the level of resistance initially encountered in the original screening of germplasm, and the adaptedness of the inbreds finally developed. Highly resistant, but unadapted, germplasm is of little use in a maize breeding programme. The resistant material that is developed must be able to compete with other adapted cultivars. These cultivars have been developed by commercial maize breeders who do not have the constraint of continually subjecting their breeding plants to severely damaging levels of insect pests.

CHAPTER 2

RESISTANCE IN MAIZE TO BUSSEOLA FUSCA

Busseola fusca has been the object of varied interest since the early 1890's. Many papers have been published (listed below) on topics such as the biology and physiology of the insect, economic significance and methods of control, as well as breeding for resistance to the insect. It is currently regarded as one of the most serious and widespread pests of maize in Africa (Jepson 1954; Walker *et al.*, 1976; Annecke and Moran, 1982; Ortega, 1987).

2.1 HISTORY AND GEOGRAPHICAL OCCURRENCE

Malley (1920) published a most comprehensive paper on all aspects of *B. fusca*. In it he discussed the biology, ecology, control measures, parasitoids and diseases. He surmised that *B. fusca* is of African origin, the larvae of which originally attacked and bored into the stems of sorghums in tropical and sub-tropical areas. Malley mentioned only 5 published records on *B. fusca* previous to 1900. He quoted a Mr. J.B. Helliar who mentioned, in his annual report to the Cape Government for 1891 that certain grubs were attacking maize plants, but the references are vague and suggest any one of cutworm, earworm or stalk borer. Helliar also mentioned the spraying (chemical not mentioned) of what appeared to be stalk borer. Various other reports in newspapers and journals during the period 1895-1900 refer to the occurrence and control of the stalk borer in the Cape Province of South Africa (Mally, 1920).

The first concise information on the life history and economics of *B. fusca* was published in 1900 by C. Fuller, Government Entomologist for Natal (Malley, 1920). That the pest was widespread and damaging is reflected in Malley's listing of over 100 references from comments at farmers' congresses to reviews in scientific publications. This was followed by a later comprehensive study on its biology by du Plessis *et al.* (1943).

From 1920 onwards, research into its biology and control gathered momentum with many researchers investigating mainly the control of the pest (Walters,1975; Walters and Drinkwater, 1975; van Rensburg and Walters, 1978; Drinkwater *et al.*, 1979 and van Rensburg *et al.*, 1980; 1988 a). It is currently under study in several African countries. For all its apparent propensity to attack maize and sorghum, *B. fusca* has several alternate hosts. Hill (1973) mentioned as alternate hosts: millet, sorghum, sugarcane, *Penisetum* spp., *Andropogon* spp., *Panicum* spp., and *Hyparrhenia* spp. However he stated that *B. fusca* is probably not capable of overwintering in these hosts, and appears almost entirely dependent on cultivated crops, mainly maize and sorghum.

2.2 GENERAL BIOLOGY

Early studies on the general biology of *B. fusca* were carried out by Malley (1920), Swaine (1957), Ingram (1958), Smithers (1960), Harris (1962) , Usua (1970 a,b), Blair (1971), and an unpublished Ph.D. thesis by Van Rensburg (1981). The more recent papers do not differ markedly from Malley's paper, and the brief description that follows of the life cycle is a synthesis from the author's own observations and other sources.

Generally, there are 2 generations a year with a partial third generation in warmer areas or during warmer autumns. Tunnels are made in the stalks at the end of the rainy season, and fully grown larvae diapause in the plant stem during winter (or the dry season in central, eastern and western African countries (Walker, 1965; Usua 1968 a, b, 1970 a, b, 1974). The larvae pupate in these tunnels at the onset of the main rainy season.

The moths emerge during spring about 3 weeks after pupation, and the mated females lay eggs on the host plant under the sheaths of the lower leaves. Varying amounts of eggs/batch and eggs/female have been recorded by different researchers.

The author recorded between 11 and 25 eggs/batch (mean of 20,6) for diapause-derived moths, and between 26-67/batch for summer-derived moths. Van Rensburg (1981) recorded a mean of 22 and 32 respectively. Malley (1920) recorded from 1-140 eggs/batch, Ingram (1958) recorded a mean of 70/batch, Harris (1962) found from 30-100/batch, and in Nigeria, Kaufman (1983) recorded 92-300/batch.

The author has recorded between 234-640 eggs/female (mean of 396/female) in laboratory cultures (diapause larvae from the field provided the source of insects). Malley (1920) recorded 891 eggs/female, Harris (1962) recorded 1000/female, Van Rensburg (1981) recorded 203/female, Ingram (1958) recorded 568/female, and Usua (1968 b) recorded 120/female for spring moths and 370 for summer-derived moths. The author recorded higher numbers of

eggs from moths derived from non-diapause larvae (up to 1057 eggs/female) which was also noted by Usua (1968 b).

Eggs hatch in 7-10 days. The larvae eat the eggshells (also noted by Kaufman (1983)) and migrate up to the funnel of the plant. Migration to adjoining plants also takes place especially assisted by wind (Jepson, 1954; van Rensburg *et al.*, 1987 a). Feeding in leaf tissue lasts for any period from 7-28 days, depending on the age of the plant at infestation. The tassel may also be attacked. Stem boring follows leaf feeding, and the development of the larvae and pupae is completed in 47-63 days within the stem (Blair, 1971). The peak emergence of the moths (what is commonly, but erroneously, termed the second generation flight) occurs in early to mid-February. These moths are attracted to late planted maize, and give rise to the second generation larvae which damage grain on the cob, and can also cause extensive stem boring damage. Most of the second generation larvae do not complete their development before the end of the growing season, and enter diapause in the drying stems.

Various papers deal with environmental and biotic influences on *B. fusca*. Generally, it has been concluded that in South Africa, the insect has a relatively poor natural enemy complex, which is rather ineffective, especially early in the season (Van Rensburg *et al.*,1987 a,b, 1988 b). Van Rensburg *et al.* recorded 9 parasitoid species, a fungus (*Beauvaria bassiana*), ants, and drowning of early instar larvae during continuous rain as causes of field mortality. He also recorded a nuclear polyhedral virus without mentioning whether it caused any mortality. Mohyuddin and Greathead (1970) recorded a far greater number of parasitoids in East Africa (20 Hymenoptera species, 6 Diptera species and a nematode). Harris (1962) also gave a full list of all predators, parasitoids and diseases recorded from cereal stem borers in Nigeria.

2.3 ECONOMIC IMPORTANCE

That *B. fusca* is considered an important maize pest is shown by the numerous publications on the control and economic significance of the insect.

Investigations on controlling the pest were carried out by Swaine (1957), Walker (1960 b), Weaving (1964) and Walker and Hodsun (1976) in East Africa. In South Africa various researchers have also investigated chemical and cultural control of *B. fusca* (Walters and Drinkwater, 1975; Walters, 1975; Van Rensburg, 1988). In Ethiopia, Gebre-amlak (1988) looked at survival of maize stalk borer in crop residues. Mlambo (1983) advised farmers in Zimbabwe to dispose of winter stover and to apply chemical control in summer. He also advocated the use of pheromone trapping in an integrated approach to control of *B. fusca*. Pheromone research was initiated in Zimbabwe (Blair and Read, 1969; Blair, 1971), and advocated by Hall *et al.* (1981) as a monitoring aid in the management of *B. fusca*. Revington *et al.* (1984) postulated that monitoring traps were a reliable guide for the farmer to improve the timing of his sprays against stalk borer. These traps are currently used in South Africa. Various estimates have been made as to the economic significance of *B. fusca* damage. Damage is caused to the leaves, grain and stems of plants. Depending however on the stage of plant growth when the pest occurs and the level of infestation, these different plant parts are damaged in different ways resulting in varying amounts of yield loss. This is more fully discussed in Chapter 5. However several researchers have attempted to put estimates to the damage. Rose (1962) distinguished between damage to the leaves and stems, but did not quantify the resulting crop loss. Van Rensburg *et al.* (1988 a) researched the injuriousness of *B. fusca* and found that the number of larvae/plant was a weak estimator of expected yield losses.

However, other researchers (Ingram, 1958; Walker, 1960 a, 1965, 1977; Harris, 1962; Rose, 1962 and Usua, 1968 a) attached special

importance to the number of larvae/plant as the damage determining factors. As will be discussed further on, studies have shown that yield loss depends on what maize variety is used, at what plant stage the infestation was initiated, and which plant part is being damaged.

Barrow (1987) showed that artificially applied first instar larvae resulted in highly significant levels of damage to leaves, stem and plant growth of several inbreds. Yield potentials of the genotypes also varied significantly under infestation, with reductions in yield ranging from 38% to 100%. Stem boring was strongly correlated with yield loss, and it appears that this type of damage is the most important.

Attempts have been made to calculate the extent of damage due to the uncontrolled feeding of the larvae in maize plants. These experiments have centered around natural infestations, which varied considerably, as did the ages of the various crops at the time of their infestation. Stalk borer infestations ranged from 14% with a yield loss of only 9.8% (Anon, 1975), to 49% stalk borer infestation with a yield loss of 37% in untreated plots (Walker, 1960 a).

Usua (1968 a) showed that the yield of a single plant could be reduced by 25% with 1-2 borers/plant, and up to 75% with 5 borers/plant. These findings differ from those of Ingram (1958) where good yields could still be obtained from plants infested with as many as 5 larvae/plant. It is possible that Usua infested a very susceptible cultivar, as he had no knowledge of the level of resistance in the germplasm he was working with. He noted that within a few days of infestation, there was evidence of dead hearts.

Wall (1967) recorded a loss of yield of 50% in a field of maize, and Jack (1917) estimated a 75% loss of yield due to second generation borers. Matthee *et al.* (1971) stated that yield losses

as high as 75% may occur as a result of *B. fusca* attack. Malley (1920) estimated the annual loss of grain to *B. fusca* in South Africa as 10%, a figure that is still quoted to this day in popular articles.

Walker (1960 b) doubled the yield on insecticide-treated plots, and Swaine (1957) obtained an 83% increase on insecticide-treated plots. Harris (1962) showed that plots treated with Endrin outyielded infested plots by 26%. However the yields recorded in this series of experiments were very low, and it appears that most of the experiments were sited on poor soil with little or no fertilizer applications. Their preliminary observations were confusing, and Harris stated that a comprehensive study of the major factors affecting yield loss was essential to the proper understanding of the losses caused by stem borers. A recent paper by Van Rensburg *et al.* (1988f) described the effect of plant population and cultivar effects on yield losses caused by *B. fusca*. They concluded that the extent of damage and degree of injury was related to the length of growing season, since the slower growing cultivars suffered the most damage. This author has concluded similarly, as discussed elsewhere in the thesis.

From the above references it is clear that the determination of yield losses is greatly dependent on many factors. These are investigated in Chapter 5.

2.4 RESISTANCE BREEDING

Before any deliberate attempt was made to develop resistant maize cultivars, several researchers remarked about the apparent naturally occurring tolerance of certain sorghum and maize cultivars. Ingram (1958) and Harris (1962) reported, in Uganda and Nigeria respectively, that sorghum appeared to have built up considerable tolerance to *B. fusca*. This is feasible because sorghum was stated by Malley (1920) to have been the original host, and may have evolved tolerance over a long period. Harris also stated that with fertile soils and good rainfall, maize appeared to be tolerant to attack, as yields were little affected by stalk borer attacks.

Previous attempts to breed hybrids resistant to maize stalk borer in South Africa relied on natural infestations in the field. The first published attempt was by du Plessis *et al.* (1943) who ascertained "that significant differences occur in the degree of stalk borer infestation of various maize varieties planted at the same time". In routine evaluation of maize cultivars at Potchefstroom, South Africa, they commented that the varieties Sahara and Peruvian carried the smallest first generation infestation, but the heaviest second generation infestation. They mentioned that Hickory King was very susceptible. However, they ascribed the differences to varietal rates of growth, and concluded that there was no resistance to stalk borer in the varieties studied.

An attempt to select for resistance was launched again in Potchefstroom in 1953 (Walters, 1974). The first results received were based on the differences in field infestation levels between different inbred lines and crosses, and seemed promising. These differences were, however, later ascribed to differences in attractiveness to female moths as sites for oviposition. At high levels of infestation, the differences tended to become obscured. This led to the supposition that such differences could not be utilized in practice, as moths were not faced with such cultivar choices in the field, but with large

plantings of the same hybrid over wide areas. The research was discontinued after the 1957/1958 season. Renewed interest arose when Kühn (1978) investigated the amounts of damage caused to several homozygous maize cultivars (inbreds) naturally infested in the field. Variable results were obtained due to moth preference, escapes, and differential numbers of eggs laid on the plants. Also at Potchefstroom, Fourie (1984) further investigated the variation in damage caused by *B. fusca* and *C. partellus* to maize genotypes under artificial infestation. He mentioned that the most significant expression of variation in resistance was the difference in plant height between the infested and protected rows. He also rated the amount of leaf damage, dead heart and ear formation. He concluded that sources of resistance to both *B. fusca* and *C. partellus* indicate a similar genetic base for resistance.

Seshu Reddy (1985) also concluded in Kenya that certain sorghum lines showed cross resistance to *B. fusca*, *C. partellus*, *E. saccharina* and *S. calamistis*. In Nigeria, Harris (1962) mentioned that preliminary studies of resistance in maize to stem borers utilizing inbreds showing resistance to *O. nubilalis* were discontinued after a few seasons. He cautioned that, in the pursuit of higher yields, factors for tolerance of and resistance to stem borers, which had been acquired by indigenous varieties through natural selection, may be bred out of improved varieties. He mentioned that high yielding maize varieties which had been introduced to Nigeria appear to be more susceptible than the locally grown varieties.

Recently, Barrow (1985) showed that different amounts of leaf damage were caused to several maize genotypes by stalk borer feeding in whorl tissue. The extent of damage was correlated with the mean larval biomass/plant, which varied in the different maize genotypes. This variation was ascribed to two resistant factors: the first is thought to be a short-lived, but effective, resistance factor in the whorl tissue which either kills or repels early instar larvae, resulting in fewer larvae feeding in

these plants. The second mechanism, operative for most of the larval feeding period in the whorl, may retard development and hence mass gain of larvae. Barrow (1987) also showed that the differences in resistance to larvae of *B. fusca* feeding in whorl tissue were effective in reducing yield loss. Yield loss was significantly correlated with leaf damage, stem boring, and plant height reduction. The resistance was found to be a heritable trait, and an extensive breeding programme is currently in progress, the results of which are presented in this thesis.

CHAPTER 3

METHODOLOGY OF BREEDING FOR RESISTANCE IN MAIZE TO *B. FUSCA*

3.1 SOURCE OF INSECTS

To sustain a HPR programme involving mass infestation and screening of tens of thousands of maize plants each season, a regular supply of hundreds of thousands of first instar larvae is required. Attempts were made over several years and at several research institutes in South Africa to artificially rear *B. fusca* on artificial diets, but without success. First instar larvae rarely survived on the various diets. Those that reached the second instar (either on artificial diet or on growing maize plants) usually successfully completed development on diets to the pupal stage. Attempts at laboratory rearing were therefore abandoned. Instead, a field collection method was used, based on planting a trap crop in early January (Barrow,1989). These plants were at a very attractive stage (knee height) in early February when the second generation moth population was active. Extensive oviposition occurred, resulting in a larval infestation of all plants. At the pre-pupal stage, larvae bored into the stems to overwinter. Several months later (July - September) they were collected as diapausing, fully grown larvae.

The maize stalks were dug out and stacked in piles awaiting manual extraction of the larvae by field workers (Plates 2,3). The stems were split open using a sturdy knife, and the larvae were carefully tipped out onto a hessian bag covering the workers legs. Larvae were then scooped up using a plastic spoon and placed into a 5l cardboard waxed ice-cream container quarter-filled with wood shavings. The shavings were first sieved to remove pieces larger than 10mmx10mm, and then put through a fine sieve to remove fine sawdust particles. These resulted in high larval mortality if left in the containers. The spoon was used to avoid high larval mortality which occurred when larvae were accidently squeezed when workers picked them up manually. In the field larvae were transferred periodically

Plate 2. Collection of overwintering larvae from fields in winter.

Plate 3. Stem splitting to obtain diapausing larvae.

during the day from the 5l containers into 100mmx15mm clear plastic petri-dishes, which were half-filled with wood shavings similar to those used in the 5l containers (Plate 4). It was observed that by completely filling the containers, larval mortality increased. This was due presumably to the tightly packed shavings either puncturing or bruising the integument during larval movement. Ten larvae were placed into each petri-dish, and these were stacked and stored in a conventional seed store cold room (unlit, 7-10 °C) for several months until the larvae were required. As the HPR programme involved the artificial infestation of tens of thousands of plants, the planting and infestation had to be spaced out over a nine-week period during summer. It was essential therefore that not all the larvae emerged from diapause simultaneously. This was achieved by controlling pupation, hence moth emergence, oviposition and the subsequent supply of first instar larvae. By holding diapause larvae under such conditions, larvae can be stored for up to 5 months if required.

In spring, the larvae were brought out of the cold room into the laboratory (temperature controlled: 24-27 °C (day) and 19-23 °C (night) and a light regime of 15:9 hrs light:dark) where they came out of diapause 30 to 50 days later, depending on how long they had been in the cold store. The longer they had been cold stored the longer they took to emerge from the diapause state and pupate. Larvae collected in early July and immediately placed in the laboratory took about 50 days for pupation to commence, while larvae cold stored for another 40 days took about 80 days to pupate. Larvae collected in late August and immediately placed in the laboratory took only about 24 days to pupate, while larvae cold stored for another 40 days took 50 days to pupate. Pupation extended over a period of at least 12 weeks after larvae were brought into the laboratory. The pupal stage lasted for 20-22 days at these temperatures, and so the petri dishes were checked for moths every 20 days, when all pupae and dead larvae were removed. Two hundred pupae were placed in each 5l cardboard waxed ice-cream container, and these containers were

Plate 4. Storage of diapause larvae in wood shavings.

Plate 5. Storage containers for pupae.

checked daily for moth emergence (Plates 5,6).

Moths were placed 20 per 5l container, and supplied with small cylindrical glass bottles (26mmx80mm) with household waxed paper wrapped spirally around the bottles as an oviposition substrate. A piece of maize leaf, approximately 100mm x 200mm was supplied as an oviposition stimulus. The absence of the leaf resulted in a marked decrease in egg laying. Initially water and sucrose were supplied, but experimentation showed this to not be a pre-requisite for successful oviposition. Unnithan (1987) found that feeding moths in a culture with sucrose in addition to distilled water shortened the pre-oviposition and oviposition period slightly, but that the moth's fecundity was unaffected. The glass bottles were removed and replaced daily.

All egg masses laid on the waxed paper (Plate 7) were stripped off by placing the paper on a table top and running a blunt knife between the wax paper and the eggs. The eggs occur in groups and, to facilitate handling and weighing, were separated from each other by washing the egg masses with tap water. All the egg masses collected on each day were placed on laboratory paper towelling fitted into a large glass laboratory funnel. Water was squirted onto the egg masses to separate the eggs and the paper was then laid flat for the eggs to dry. After a few hours, the eggs were brushed off, and separated into 600mg lots. The eggs were kept in small open ended glass bottles (10mmx50mm, each containing 600mg eggs). These were kept at 31-34 °C (day) and 19-23 °C (night) in 5l cardboard containers supplied with a wad of water-soaked cotton wool to maintain a high relative humidity inside the container. When the eggs reached the black head stage after 5-6 days, the bottles were plugged with cotton wool stoppers to prevent larval migration after eclosion.

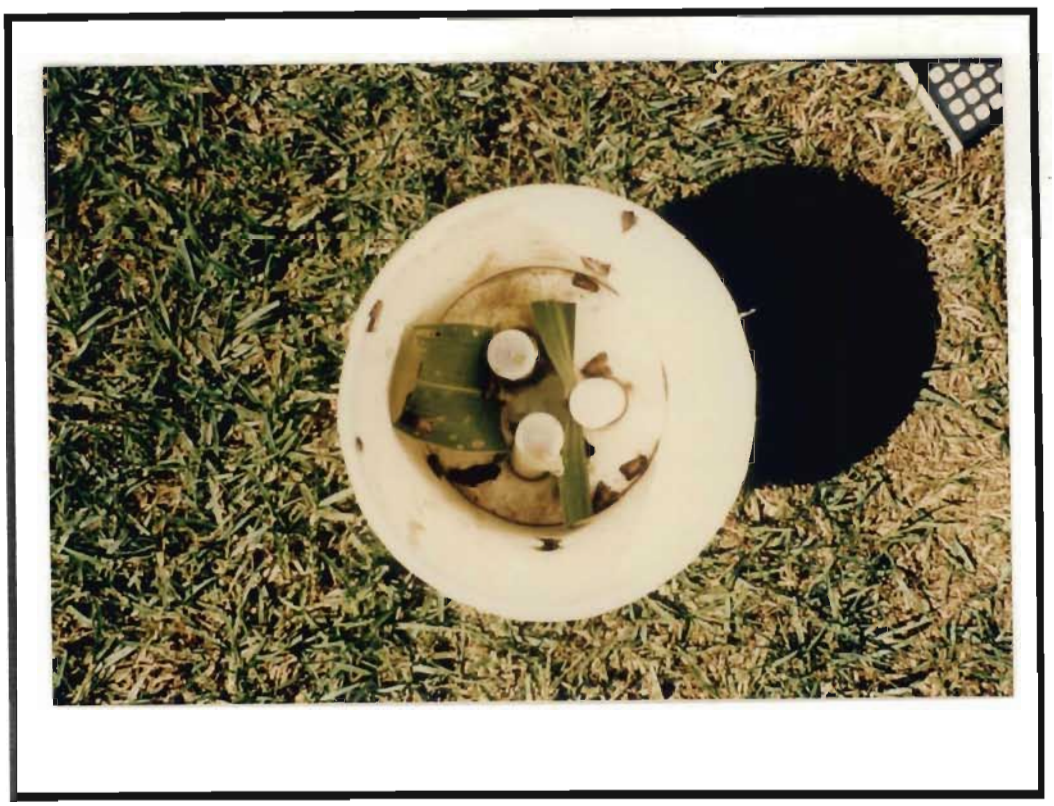


Plate 6. Moth oviposition containers.

Plate 7. Egg masses from laboratory rearing of *B. fusca*.

3.2 INFESTATION METHODS

As the natural oviposition site is under the sheath of the lower leaves, it was appropriate to initially attempt artificial infestations by placing egg masses at this site. Egg masses were cut into groups of *ca.* 20 eggs, and affixed onto pieces of water soluble glue-backed strips of paper. They were incubated to the black head stage, and then each plant to be infested received one egg "stamp". These stamps were placed just under the leaf sheath exactly where natural oviposition occured. This was a time consuming procedure, unsuited to mass infestations, only suitable for the initial stages of the research programme. For this reason the method was abandoned and a "bazooka" utilized for mass screening (Plate 8). This is a hand-operated device developed by Mihm (1983), which delivers a pre-determined number of neonate larvae into the plant whorl.

The "bazooka" utilized a mixture of larvae maize meal. Within one day after larval eclosion, 600mg of the larvae + egg shells were thoroughly mixed with 100g of maize meal (sifted to remove the very fine powdered maize meal which caused high larval mortality). The mixture was poured back and forth several times through a glass funnel (plastic funnels were avoided as they set up static) into 1l glass laboratory beakers. After about 20 such mixings, the "bazooka" was filled with the mixture and the calibration was checked. The pre-determined mass of 600mg of larvae (containing *ca.* 6000 larvae) plus 100g of maize meal ensured that 2 doses delivered by the "bazooka" into each plant funnel resulted in 0,33g of mixture/plant. This quantity introduced between 16-22 larvae/plant if the larvae and maize meal had been thoroughly mixed (See 4.1.1., 5.1.1. and 5.1.2.). This number of larvae was found to give optimal damage expression, and to allow maximal utilization of neonate larvae. The calibration consisted of delivering two doses into each of ten glass petri dishes, and then counting the larvae delivered into each dish. Once the mean of 10 petri dishes was about 20 larvae per dish (± 3 larvae deviation allowance per petri dish), then field infestations commenced.

Plate 8. Application of neonate larvae with "bazooka".

All maize plants were infested when they reached a height of about 35cm with two doses each of about 8-11 first instar larvae. This gave a total of 16-22 first instar larvae per plant. Larger numbers of larvae often resulted in such severe damage to the developing tassel that no pollination was possible, or to the stem tissue that no grain developed. Fewer than this amount of larvae resulted in too many apparently "resistant" plants for meaningful selection. Attempts to introduce black head stage eggs into the plants instead of first instar larvae were unsuccessful, presumably due to the presence of low relative humidity having a detrimental effect on successful larval eclosion.

Plants were spaced 45cm apart in the row, with 10 plants normally planted per row, and rows planted 90cm apart giving a population of 25000/ha. Occasionally seeds were planted every 22,5cm to get 20 plants per row. Depending on the material to be infested, either 6 out of 10, 10 out of 10, or 20 out of 20 plants were infested. Where inbreds were screened for resistance, only 6 out of 10 plants were infested, and the remaining 4 plants were used for stunting comparisons. This had a secondary objective of obtaining seed at harvest in case the infested plants were so badly damaged that they yielded no grain. Where segregating material was planted, all the plants in the row (normally 10) were infested so that the more resistant plants could be selected. Where populations or composites were to be screened for the first time, all 20 plants in a single row were infested. An impression was gained on the level of resistance present in each population. Those populations that showed a higher than average level of resistance were then planted out in greater quantities the following season. Development of resistant germplasm was then commenced by utilising different plant breeding procedures.

3.3 DAMAGE EVALUATION

As larvae feed on different parts of the maize plant, it was an unrealistic goal to attempt to obtain resistance in each of these feeding sites. Because larvae feed predominantly in the whorl tissue (Plate 9), attempts to identify sources of resistance centered around this particular site.

Barrow (1985) noted that rating leaf damage after 21-25 days feeding on a scale of 1 (minimal damage) to 5 (severe leaf shredding) was a quick and efficient field method of whorl damage assessment (Table 3.1.) (Plates 10,11).

Table 3.1. Leaf feeding rating scale (damage rated after 21-25 days feeding)

Class	Description
1.	Small pin or fine (up to 2mm diameter) shot-hole injury - few in number and sparsely distributed on upper leaves.
2.	Numerous and widely distributed small perforated shot-holes (up to 2mm diameter).
3.	Large slightly elongated shot-holes (up to 5mm diameter and 15mm length) widespread over leaf area.
4.	Large shot-holes merging into elongated lesions (up to 8mm diameter and 30mm length).
5.	Large lesions (up to 8mm diameter and 50mm length) on all leaves causing leaf tatters and shreds. Occasionally growing point killed after extensive leaf feeding.

1-3 Acceptable for utilization in HPR programme.

4-5 Unacceptable for HPR programme.

Any feeding period shorter than 21 days did not allow sufficient time for discernible differences in leaf damage to occur. Rating whorl damage in quick maturing cultivars later than 25 days can often run into problems with tassel emergence (see 5.1.2.). As the tassel emerges from the whorl, larvae cease feeding on leaves and either feed on the developing tassel, or

Plate 9. *B. fusca* larvae feeding in maize whorl.

(1)

(2)

(3)

Plate 10. Leaf damage ratings 1 - 3 (Useful in development of resistant germplasm).

(4)

(5)

Plate 11. Leaf damage ratings 4 & 5 (Too susceptible to utilize in development of resistant germplasm).

migrate out of the enclosed tassel into the stem. Comparisons of leaf damage between cultivars (especially of different maturities) was therefore difficult if ratings were carried out after 25 days feeding. With this rating system only plants rated 1-3 were considered worthwhile selecting at harvest.

Damage ratings for inbred lines were taken on each of 6 infested plants out of the row of 10 plants. Where uninfested control plants were available for stunting comparisons, a stunting rating on a scale of 1 (minimal stunting) to 5 (severely stunted) was taken in addition to a leaf damage rating (Plate 13). Note was also taken at harvest on the extent of stunting of the stem between the tassel and ear of each plant, and also of the ear size in making selections.

For segregating material, all plants in the row were infested, rated individually, self pollinated, and the most resistant ones selected at harvest. Although assessment of stem feeding (see plate 12.) (by splitting stems and rating the damage) forms part of resistance breeding, the labour required to obtain such results was too great for the method to be used for regular field assessment. However, investigations were carried out on stem damage as this type of damage is an integral part of the insect-plant interaction (see 5.2.). Also, the size of the ear was often an indication of how much sap flow interference there had been due to stem tunneling. Earlier work showed that yield loss in several inbreds was significantly positively correlated with the amount of stem boring ($r=+0.56$, $P<0.01$); plants that showed severe stem boring also showed significant reductions in plant height ($r = +0.73$, $P<0.01$) Barrow (1987).

It was concluded that field selection for resistance to *B. fusca* should rely on leaf damage recorded after about 24 days feeding and visual assessment at harvest of plant height reduction and yield.

Plate 12. Severe stem boring by *B. fusca* larvae.

Plate 13. Severely damaged maize plants.
Uninfested control plants in background.

3.4 STATISTICAL METHODS

The various data were analysed with the GENSTAT (version 4.3) system of Rothamsted Experimental Station (U.K.), on a Univac / Sperry Computer of the University of Natal. The form of the analyses varied between the analysis of Randomized Complete Block Design (RCBD) to RCBD split for the factor time - i.e. plots were repeatedly measured over selected time intervals (Split Plot).

Statistical results are compared at the 5% and 1% levels of significance. These are designated variously by $P<0.05$ (or *) for the 5% level, and $P<0.01$ (or **) for the 1% level. Individual comparisons between the specific treatments (treatment combinations) are made using the t-test, usually in the form of Least Significant Differences (LSD). In the discussion following the ANOVA tables, all data are compared and discussed at the 5% level for realistically applicable separation of differences.

In some instances due to the skewness / heterogeneity of the data, it was found necessary to transform the data using the logarithmic (Log_{10}) transformation to standardize the variance and improve the degree of approximation of the Analysis of Variance (ANOVA).

CHAPTER 4

EFFECT OF RESISTANCE ON *B. FUSCA*

In any HPR breeding programme, it is essential that adequate techniques are available for measuring the degree of resistance expressed by the plant towards the insects. This necessitates assessment of either the effect of the plant on the insect, or of the insect on the plant. The effects of *B. fusca* on the plant are discussed under Chapter 5, and the effects of the plants on *B. fusca* are discussed here. Dahms (1972 a) listed several criteria used to evaluate resistance. Some criteria measured only one factor (e.g. number of insects recorded at any one time). Others measured the combined effect(s) of all factors (i.e. crop yield loss under infestation). Concerning the effect of the plants on the insects, Dahms mentioned the following criteria: (i) determination of the number of insect stages attracted to a cultivar when given a free choice, (ii) observation of the comparative effects of forced feeding (confinement) on plants or cultivars by measuring numbers of insects surviving, length of insect life cycle, mortality, reproductive rates, moulting etc., (iii) weighing of insects after definite feeding periods on different cultivars, (iv) determination of the number of eggs oviposited, (v) determination of the number of surviving insects and progeny produced, (vi) determination of the effect of plant parts on behaviour or orientation of the insect, (vii) reproduction potential of insects fed various plant diets containing different plant cultivars.

As *B. fusca* moths orientate towards and oviposit on the crop in the field, an appropriate starting point was to investigate oviposition on different maize genotypes. During the summer of 1978 weekly counts were taken of the number of egg masses laid on several hundred inbreds planted out in the conventional maize breeding programme. There were major differences in what was assumed to be attractiveness/repellence of some inbreds to

the moths. Some inbred lines were far more attractive than others to the moths and had up to three egg masses per plant (300% infestation). Others had a 0% infestation. During the summer of 1979, blocks of these previously sampled inbreds were planted out in late October so as to be at an attractive stage in late November when the wild moth population was flying. In most cases there were highly variable results. Inbreds that had previously showed a 0% infestation now showed up to 70% infestation, whereas others that had shown 100% infestation now showed as low as 10% infestation. Blocks of the same inbreds that were planted at weekly intervals to give a range of ages also showed a definite moth preference for certain ages and heights of plants over others. It was evident also that preference for oviposition on some inbreds depended on the attractiveness or repellence of neighbouring inbred lines. If inbred A had attractive (to moths) inbreds B and C on either side in the field, a 0% infestation would be recorded on A, with high percentage infestations recorded on B and C. However if A was planted next to unattractive inbreds D and E, a high % infestation occurred on A.

This line of research was thus abandoned for the following reasons:

(i) Screening of inbreds for moth resistance would necessitate the planting out of hundreds of different inbreds in adjacent rows. As described above, this would lead to preference for some inbreds over others. This situation could change the following season or in the same season if the spatial arrangement of inbreds was altered, thus giving conflicting data.

(ii) Moth preference for a certain developmental stage of maize, normally correlated with height, is well documented (van Rensburg, 1981, 1987 b). Sequential weekly plantings are required in a breeding programme for even work distribution during the season. Moths would be attracted to certain plantings, a situation which would change weekly, resulting in confusing data.

(iii) Natural populations of wild moths would have to be used, as there would be no way of carrying out a sufficiently large number of screenings in the field with artificial release of moths in cages. Controlled infestations, so essential to any HPR programme, would therefore not be possible.

(iv) During the first generation flight in mid-summer when most of the screening work would be done, oviposition in commercial fields rarely rises above 30% of the plants receiving egg masses. This would be far too low a level for successful screening of maize germplasm.

(v) Moth preference or avoidance for certain inbreds could be due to chemical orientation to the height or growth stage of those inbreds. These inbreds are eventually combined in hybrids, which consist of 2, 3 or 4 inbreds, and heterosis is expressed as hybrid vigour and increased height. The individual effects of the inbreds would therefore be masked and the resistance probably altered.

(vi) The final testing of any moth resistant inbreds or hybrids would require the planting out of many plants in large blocks. These plants would then have to be assessed for natural oviposition and compared to oviposition in control blocks. The results would be dependent therefore on the relative attractiveness of plants in the control block compared to plants in the resistant block. Moths may be totally attracted to the control blocks and not merely repelled by the resistant block. Also, if the moths had only the resistant maize to oviposit on because no other hybrid was planted in the area, would they be forced to oviposit in this maize from lack of any choice? This type of assessment would be totally impractical. How much better surely to accept oviposition in the crop, and now, having a captive audience of larvae so to speak, attempt to develop resistance to these larvae which have limited mobility.

To elucidate the inter-relationship of borer and plant, investigations were carried out using artificial infestation of plants with neonate larvae. These investigations included the effects of leaf, stem and tassel resistance on various aspects of *B. fusca* survival, namely (i) numbers of borers found in the plants after prescribed periods of feeding, (ii) larval mass and biomass after prescribed feeding periods, and (iii) development and viability of life stages during, and consequent to, larval feeding.

All the experiments described in this thesis were planted at "Hildesheim", Pioneer Seed Company's Research Farm in Greytown, Natal, South Africa. Various fields were used over the years, but all were basically prepared as for commercial plantings of maize. Unless otherwise stated, all the seeds were planted from late October to early December in order to avoid the plants being at an attractive stage in mid- to late- November when the wild population of *B. fusca* was ovipositing. This prevented any natural oviposition on the experimental plants which may have confused damage assessment. Seeds were initially planted two per hill, 22cm apart, and were then thinned to single plants when the plants were 10cm tall. This resulted in a population of approximately 50 000 plants/ha in rows 0.9m apart.

4.1 LEAF RESISTANCE

The initial years (1979 to 1981) of screening maize germplasm for possible resistance to *B. fusca* involved artificially infesting Pioneer Seed Company's wide range of standard inbred lines, and assessing leaf feeding damage after 21-28 day's feeding. This was done to identify material that showed very little damage and therefore presumably some form of resistance to *B. fusca*. Infesting and rating methodologies were also continually improved over the years to produce as wide a range of leaf damage as possible.

During the 1979/80 season, artificial infestation of inbred lines with neonate larvae resulted in clearly defined differences in leaf damage, caused by larvae feeding deep down in the whorl. These differences were further investigated during the 1980/81 season, where replicated data were obtained. In addition, several single cross hybrids containing some of these previously screened resistant and susceptible inbreds were also artificially infested. Compared with the leaf damage ratings of their constituent inbred parents, the observed leaf damage ratings of each hybrid were indicative of heritability of resistance / susceptibility. The resistance was presumed to be an additive mechanism that reduced insect feeding, indicating an antibiosis type of resistance. To investigate further the relationship between leaf feeding and borer, a series of experiments was carried out from 1981 to 1989.

4.1.1 Numbers, mass and biomass of larvae feeding in maize

4.1.1.1 Larval survival and development in different maize genotypes subjected to various infestation levels

Economic and efficient usage of larvae is an essential component in any HPR programme. In 5.1.1. it was determined that infestation of approximately 20 larvae/plant was the most efficient infestation level to utilise in screening genotypes. This level gave the widest range of leaf damage values between maize genotypes, and did not result in great wastage of larvae.

The following experiment was designed to investigate larval survival in genotypes receiving various infestation levels. Larval survival was assessed in 4 inbreds and 2 single cross hybrids receiving different larval infestation levels.

(i) Materials and methods

The four maize inbreds chosen for the experiment had previously been screened with many others for resistance to first instar larvae. The two inbreds D57 and MO6 had shown a resistant leaf reaction to larvae feeding in the whorl tissue, and the inbreds 56 and 58 had shown susceptibility. Two single cross hybrids containing these inbreds had also been previously screened. D57 x MO6 showed a more resistant leaf damage reaction than 56 x 58. The experiment was carried out in a commercial maize field and the seeds were planted on 19th October 1981 with plant emergence on 29th October 1981.

A randomised complete block design was used, with split plots and 3 replications. The whole plots were the cultivars, and the sub-plots were five different larval infestation levels. Each row contained 20 plants.

Each cultivar (whole plot) was planted in a block of 10 rows, each therefore containing 200 plants. For each cultivar, the 10 rows were split into five two-row subplots (40 plants). The plants in each sub-plot received one of the following mean larval treatments: 4.2; 8.9; 13.4; 17.2; and 33.8 larvae/plant (representing desired treatment levels of 5, 10, 15, 20 and 30 larvae/plant). All plants were infested with neonate larvae applied with a "bazooka" 35 days post emergence on 5th December 1981.

Maize plants had been previously found to be sensitive to applications of the maize meal. Symptoms similar to sun scorch had appeared on the leaves at the area of contact with the maize meal. To avoid too much maize meal being applied to the whorl, instead of calibrating the applicator at 5 larvae per

application, and then putting 2 "shots" into each plant for the 10 larvae application and progressing to 6 "shots" for the 30 larvae application, the "bazooka" was calibrated separately for each larval application. This explains why the dosages were not exactly 5, 10, 15, 20 and 30 larvae per treatment as intended. The whorls of 40 plants in each sub plot were removed from the field 28 days after infestation. The larvae feeding in each plant were counted and weighed individually, and the mean values calculated.

(ii) Initial findings and infestation level

Table 4.1.1 Mean leaf damage ratings for five infestation levels, after 28 days feeding, averaged over 6 cultivars

INFESTATION LEVEL (larvae/plant)				
4.2	8.9	13.4	17.2	33.8
2.0a[1]	2.7b	3.1c	3.5d	3.5d

[1] **Means in columns followed by the same letter are not significantly different at the 5% level**

L.S.D. (5%) = 0.31

Increasing the number of larvae per plant to beyond 17 resulted in no greater plant damage. These data were confirmed in other experiments reported elsewhere (see 5.1.1., 5.1.2.). Also, as field observations indicated that a mean of approximately 20 eggs/plant were found under natural conditions, all field infestations from here on were carried out with the "bazooka" calibrated to *ca.* 20 larvae/shot.

(iii) Results and discussion

The effect of the different cultivars on the borers was assessed by pulling out the whorls and counting and weighing the larvae found feeding therein.

(a) Numbers of larvae

There were significant ($P<0.05$) differences between the numbers of larvae recovered from each cultivar (Table 4.1.2.). There were also highly significant differences ($P<0.01$) between the numbers of larvae recovered from each infestation level. The interaction was also highly significant ($P<0.01$).

Table 4.1.2. Significance, on mean numbers of larvae/plant recovered after 28 days feeding, of 5 different levels of stalk borer larvae applied to 6 maize cultivars

SOURCE OF VARIATION	F	F distribution values	
		5 %	1 %
Cultivars	3.74*	3.33	5.64
Infestation levels	67.77**	2.57	3.75
Cultivar x infestation levels	36.59**	1.81	2.33

C.V.% Whole Plots = 8.3%
Sub-plots = 17.7%

The effect of cultivars on numbers of larvae/plant

The mean numbers of larvae/plant recovered from each cultivar are shown in Table 4.1.3.

Table 4.1.3. Mean numbers of larvae/plant recovered from 6 maize cultivars after 28 days feeding, averaged over 5 larval infestation levels

CULTIVAR					
M06	D57	D57xM06	58	56	56x58
2.19a[1]		2.19a	2.25a	2.41ab	2.91bc3.34c

[1] Means followed by the same letter are not significantly different at the 5% level

L.S.D.(5%) = 0.61

As expected M06, D57 and their single cross M06 x D57 had significantly fewer larvae than 56 and 56 x 58. This suggests a resistance mechanism affecting survival of larvae which results in fewer larvae. The inbred 58 had as few larvae as the more resistant (leaf damage) cultivars. This indicates the existence in this inbred of a resistance mechanism affecting numbers of larvae, but, as will be discussed below, not one affecting larval mass. It is also apparent that these cultivars exhibited a continuous range of resistance which affected survival of larvae, thus indicating an additive type of resistance. It appears that the resistance is a heritable trait, as evidenced by the similarity of data in the single cross D57 x M06 and the constituent parents D57 and M06.

The effect of infestation levels on numbers of larvae

Infestation levels had a far more highly significant ($P<0.01$) impact than cultivars on numbers of surviving larvae. The mean numbers of larvae recovered from each infestation level after 28 days feeding are shown in Table 4.1.4.

Table 4.1.4. Mean numbers of larvae/plant recovered from 5 infestation levels, after 28 days feeding, averaged over 6 maize cultivars

INFESTATION LEVEL (larvae/plant)				
4.2	8.9	13.4	17.2	33.8
0.50a[1]	1.02a	2.24b	3.76c	5.26d

[1] Means followed by the same letter are not significantly different at the 5% level

L.S.D. (5%) = 0.90

More larvae were recovered as the infestation levels increased. However, larval death and migration resulted in low percentage recoveries from all levels.

The effect of cultivar x infestation levels interaction on numbers of larvae

The interaction between these two variates is shown in Table 4.1.5. and Fig.1.

Table 4.1.5. An interaction table showing the mean number of larvae/plant from 6 maize cultivars receiving 5 infestation levels, removed after 28 days feeding

	INFESTATION LEVEL (larvae/plant)				
CULTIVAR	4.2	8.9	13.4	17.2	33.8
M06	0.30a[1]	0.68c	1.74e	2.74h	5.51l
D57	0.31a	0.80c	1.72e	3.01h	5.13kl
D57xM06	0.42a	1.23c	1.71e	3.69h	4.54k
58	0.30a	0.80c	2.30ef	3.23h	5.31kl
56	0.79a	1.10c	2.79fg	4.81i	5.07kl
56x58	0.91a	1.52c	3.20g	5.08i	6.00l
Range (max.-min.)	0.61	0.84	1.48	2.34	1.46

[1]Means in columns followed by the same letter are not significantly different at the 5% level

L.S.D. 5%	Main effect	Interaction
Cultivar	0.61	1.73
Infestation level	0.90	0.87

The interaction between cultivars and infestation levels was highly significant ($P<0.01$). Increased infestation levels (with the exception of the 33.8 level) resulted in an increase

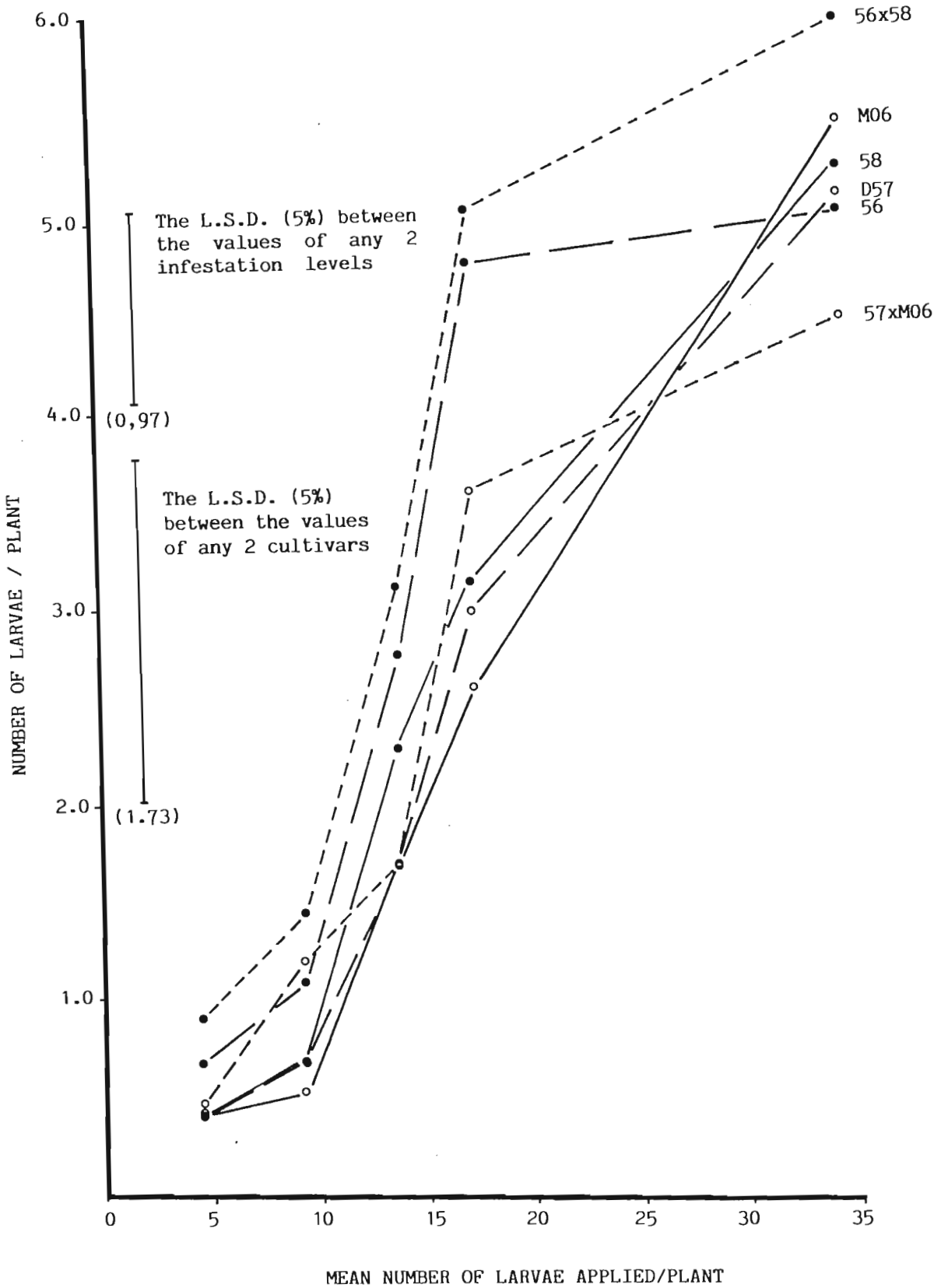


Fig.1. Mean number of larvae per plant removed after 28 days feeding in 6 maize cultivars receiving 5 infestation levels

in the range between the highest and lowest values being recorded from all cultivars. This strong correlation between infestation level and larval survival complicates further the insect-plant interaction and the assessment of damage in the search for resistance. It also highlights the importance of correctly calibrating the "bazooka" in all screening of maize genotypes. At the 4.2 larvae/plant level, there was a 300% increase in numbers of larvae recovered from 56x58 compared with the numbers recovered from M06 and 58. This difference however was not significant. There was also no significant difference in numbers of larvae recovered from cultivars at the 8.9 larvae/plant level. In contrast, at the 13.4 and 17.2 larvae/plant levels, there were large and significant ($P<0.05$) differences between two groups: (M06, D57, D57xM06, 58) and (56, & 56x58). At the 33.8 level, differences were not so distinct. Only the single cross D57xM06 showed significantly ($P<0.05$) fewer larvae than 56x58 at the 33.8 level.

Application rates of 13.4 and 17.2 larvae/plant therefore showed up one of the most critical requirements in an HPR programme, namely the greatest range in larval numbers between cultivars.

It is interesting to note the percentage increases in larval recovery from each cultivar as the infestation level increases:

Table 4.1.6. Percentage recovery of larvae from each cultivar over the different larval treatments, after 28 days feeding

	INFESTATION LEVEL (larvae/plant)					
Cultivar	4.2	8.9	13.4	17.2	33.8	Mean
M06	7.1	7.8	12.6	15.7	16.3	13.5
D57	7.1	8.9	12.6	17.4	15.0	14.1
D57xM06	9.5	13.4	12.6	21.5	13.3	14.8
58	7.1	10.1	17.2	18.6	15.6	15.5
56	19.0	12.3	20.8	27.9	17.7	20.0
56x58	21.4	16.8	23.9	29.6	17.7	22.6
Mean	11.9	11.6	16.7	21.8	15.5	15.5

Of the cultivars, 56 and 56x58 showed a much higher % mean larval recovery, and for all levels of infestation except 8.9 larvae/plant. This indicates the lack in these cultivars of a resistant mechanism which affects numbers of larvae.

Increasing the numbers of larvae up to 17.2 larvae/plant resulted in greater % recoveries. The exception was at the high application rate of 33.8 larvae/plant, which resulted in the recovery of only 15.5% for all cultivars. This was possibly caused by crowding, resulting in either death or larval migration out of the plant during the feeding period in the whorl (larval migration is discussed in 4.1.1.5.). This information, coupled with data obtained on leaf damage (5.1.1. and 5.1.2.) led to the decision to use the maximally efficient and most economic infestation level of between 16-22 larvae for routine screening of maize germplasm.

(b) Larval mass

In addition to a resistant mechanism reducing numbers of larvae by causing larvae to either die (antibiosis) or migrate out of the plant (repellence), it was thought that other mechanisms may also influence the insects' successful exploitation of the plants as a food source. One effect in particular was reduced larval mass gain. Larvae were therefore removed and weighed.

There were highly significant ($P<0.01$) differences between the mean larval mass of larvae removed from each cultivar at each of the different infestation levels.

Table 4.1.7. Significance, on mean larval mass recovered after 28 days feeding, of 5 different levels of larvae applied to 6 maize cultivars

SOURCE OF VARIATION	F	F distribution values	
		5%	1%
Cultivar	157.63**	3.33	5.64
Infestation levels	1467.33**	2.57	3.75
Cultivar x infestation levels	34.98**	1.81	2.33

C.V.% Whole Plots = 12.9%
Sub-plots = 19.8%

<u>The effect of cultivars on larval mass</u>

The mean larval mass recovered from each cultivar is shown in Table 4.1.8.

Table 4.1.8. Mean larval mass (mg) of larvae recovered after 28 days feeding from 6 maize cultivars, averaged over 5 infestation levels

CULTIVAR					
M06	D57	D57xM06	56	58	56x58
59.80a[1]	69.16ab	76.97b	114.44c	118.09c	123.39c

[1] Means followed by the same letter are not significantly different at the 5% level

L.S.D. (5%) = 14.15

The data show the M06/D57 group as having the greatest effect on larvae. This is similar to the previous conclusion on resistance affecting numbers of larvae. Heritability of the resistance was also in evidence. Larvae recovered from 56x58 were just over double (106,34%) the mass of larvae recovered from M06. As will be discussed later, there are two separate mechanisms involved in resistance to stalk borer larvae feeding in whorl tissue. One affects numbers of larvae and the other affects larval growth and mass gain. Data presented elsewhere also suggest the possibility of linkage of the two mechanisms. It appears here that the D57/M06 group had both mechanisms, while 58 had only the one affecting numbers of larvae, while 56 and 56x58 had neither mechanism.

The effect of infestation levels on larval mass

As with numbers of larvae, infestation levels had a more significant effect on mean larval mass than did cultivars. The mean larval mass resulting from each level of infestation is shown in Table 4.1.9.

Table 4.1.9. Mean larval mass (mg) of larvae recovered after 28 days feeding from 5 infestation levels, averaged over 6 maize cultivars

INFESTATION LEVEL (larvae/plant)				
4.2	8.9	13.4	17.2	33.8
122.76a[1]	105.13b	99.05b	80.54c	60.73d

[1] Means followed by the same letter are not significantly different at the 5% level

L.S.D. (5%) = 12.27

There was a large decrease in larval mass as the infestation level increased. Larvae that were recovered from plants receiving 4.2 larvae/ plant weighed just over double the mass of larvae recovered from plants receiving 33.8 larvae/plant. This result is highly significant ($P<0.01$). The data from Table 4.1.4. showed a significant increase in larval numbers recovered after 28 days feeding as infestation levels increased. Only a mean of 0.50 larvae/plant were recovered from the 4.2. larval level, increasing steadily over levels to 5.26 larvae/plant for the 33.8 larval level. Evidently, larval crowding resulted in either physical or chemical interference between larvae. This resulted in reduced successful establishment of larvae in those plants receiving higher infestation levels. This again stresses the importance of correct calibration of infestation, and also introduces a further variable in the plant/insect interaction.

<u>The effect of cultivar x infestation levels interaction on larval mass</u>

The interaction between these two variates is shown in Table 4.1.10. and Fig.2.

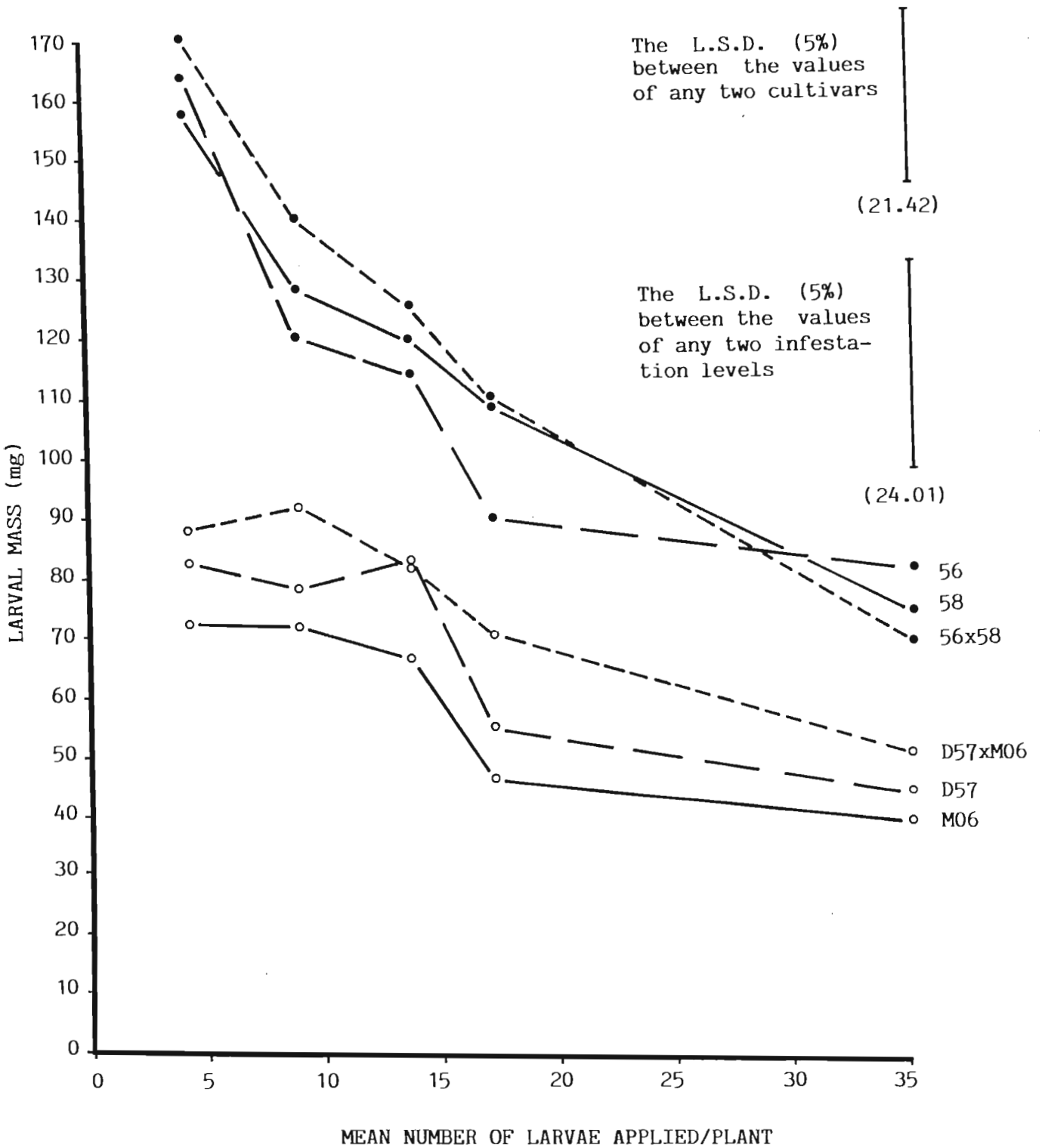


Fig.2. Mean larval mass of larvae removed after 28 days feeding in 6 maize cultivars receiving 5 infestation levels

Table 4.1.10. An interaction table showing the mean larval mass (mg) of larvae recovered after 28 days feeding from 6 maize cultivars receiving 5 infestation levels

	INFESTATION LEVEL (larvae/plant)				
CULTIVAR	4.2	8.9	13.4	17.2	33.8
M06	72.33a[1]	71.49a	67.63a	47.31a	40.26a
D57	83.00a	78.20a	83.21a	56.21a	45.19a
D57xM06	88.56a	92.64a	82.43a	70.35ab	50.89ab
56	164.30b	120.13b	114.66b	90.66bc	82.44c
56x58	170.17b	140.32b	125.97b	110.04c	70.48bc
58	158.20b	128.00b	120.42b	108.71c	75.13c
range:	97.84	68.83	58.34	62.73	42.18

[1]Means in columns followed by the same letter are not significantly different at the 5% level

L.S.D. 5%	Main effect	Interaction
Cultivars	14.15	21.42
Infestation level	12.27	24.01

The interaction between cultivars and infestation levels was highly significant ($P<0.01$).

There was a gradual reduction in the range observed between the highest and lowest values in each infestation level. At the lowest level of 4.2 larvae/plant, the greatest mass was recorded for larvae feeding in 56x58 (170.17mg). This value was 97.84 mg heavier (or 135.27%) than that of larvae feeding in M06

(72.33mg). At the 33.8 larvae/plant level the difference in mass between larvae feeding in 56 and M06 had dropped to 42.18 mg (104.77% heavier larvae in 56). Fig. 2. illustrates clearly the grouping of data into the two genotype groups.

Between infestation levels for each cultivar, there were similar reductions in larval mass. These reductions were most pronounced for the 56/58 cultivars (see Fig.2). For each of M06, D57 and D57xM06 there were no significant differences between mean larval mass recorded from infestation levels of 4.2, 3.9 and 13.4 larvae. Significant differences began to appear at the 17.2 level, when compared to the 4.2 and 8.9 levels, with no significant differences between the 17.2 and 33.8 levels.

The 56/58 genotypes showed very high larval mass for the 4.2 larval infestation level. These values varied significantly from values at the 8.9 and 13.4 larval levels. There were no significant differences between the 8.9 and 13.4 levels. There were also no significant differences evident between the 13.4 and 17.2 levels, but there were significant differences evident between the 8.9 and 17.2 levels. A further significant drop in larval mass occurred in 56x58 and 58 between the 17.2 and 33.8 larval levels.

In addition to a resistant mechanism affecting numbers of larvae, the D57/M06 group had an antibiotic effect on larval growth. It would appear that the 56/58 group lacked this mechanism.

(c) Larval biomass

As it is the biomass of larvae (number of larvae/plant x mean larval mass) feeding in the plant whorl that causes the damage, an Analysis of Variance was carried out on the larval biomass recovered from each cultivar. There were highly significant differences between the mean larval biomass/plant removed from each cultivar, and from each of the infestation levels. The interaction was also highly significant:

Table 4.1.11. Significance, on mean larval biomass/plant (mg) recovered after 28 days feeding, of 5 different levels of stalk borer larvae applied to six maize cultivars

SOURCE OF VARIATION	F	F distribution Values	
		5%	1%
Cultivar	215.66**	3.33	5.64
Infestation levels	1986.34**	2.57	3.75
Cultivar x Infestation levels	107.59**	1.81	2.33

C.V. % Whole Plots = 13.6%
Sub-plots = 20.5%

The effect of cultivars on larval biomass

The mean larval biomass recovered from each cultivar is shown in Table 4.1.12.

Table 4.1.12. Mean larval biomass/plant (mg) recovered after 28 days feeding from 6 maize cultivars, averaged over 5 infestation levels

CULTIVAR					
M06	D57	D57xM06	58	56	56X58
107.88a[1]	126.48ab	156.54b	235.38c	287.17d	350.62e

[1] Means followed by the same letter are not significantly different at the 5% level

L.S.D. (5%) = 37.38

The mean larval mass contributed more to biomass than mean numbers of larvae/plant. The differences in biomass between

hybrids were significantly ($P<0.01$) far greater than the differences due to numbers or mass alone. The effect of M06 having both resistant mechanisms is clear when compared with 56x58, which has neither resistant mechanism.

The effect of Infestation levels on larval biomass

The mean larval biomass recovered from each infestation level is shown in Table 4.1.13.

Table 4.1.13. Mean larval biomass/plant (mg) recovered after 28 days feeding from 5 infestation levels, averaged over 6 maize cultivars

INFESTATION LEVEL (larvae/plant)				
4.2	8.9	13.4	17.2	33.8
69.45a[1.]	112.16a	233.62b	317.43c	320.74c

[1.] **Means followed by the same letter are not significantly different at the 5% level**

L.S.D. (5%) = 49.76

Although larval mass decreased significantly over infestation levels (Table 4.1.9.), an increase in numbers of larvae with increasing infestation levels resulted in an increase in larval biomass over infestation levels. There was no significant increase from the 4.2 to 8.9 level, but the increases from 8.9 to 13.4 and from 13.4 to 17.2 were significant. No significant increase in biomass was recorded when larval infestation was increased from 17,2 to 33.4 larvae/plant.

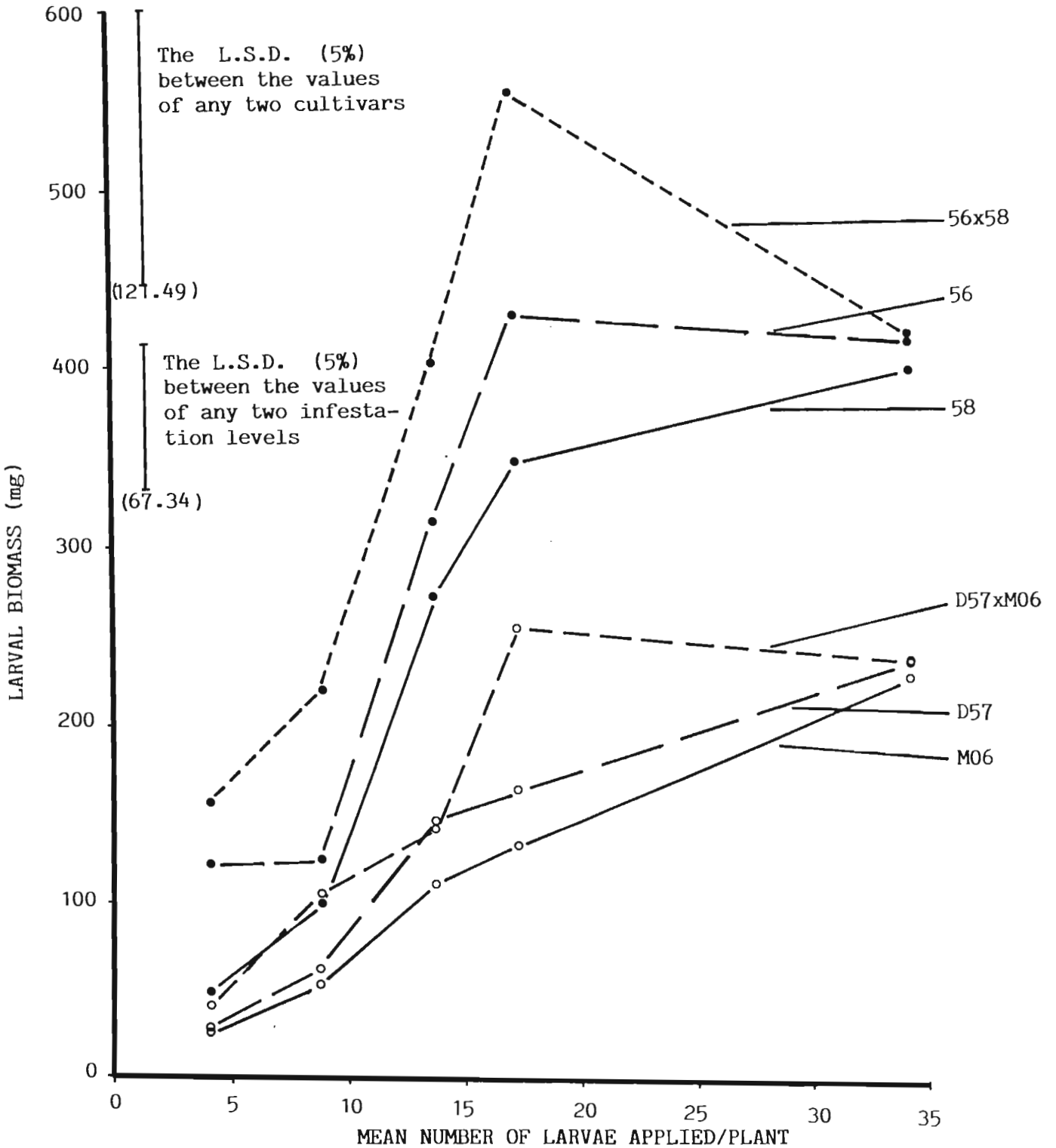


Fig.3. Mean larval biomass/plant (mg) removed after 28 days feeding in 6 maize cultivars receiving 5 infestation levels

The effect of cultivar x infestation levels interaction on larval biomass

This interaction is shown in Table 4.1.14 and Fig. 3.

Table 4.1.14. An interaction table showing the mean larval biomass/plant (mg) recovered after 28 days feeding from 6 maize cultivars receiving 5 infestation levels

	INFESTATION LEVEL (larvae/plant)				
CULTIVAR	4.2	8.9	13.4	17.2	33.8
M06	21.69a[1]	48.61a	117.67a	129.63a	221.83a
D57	25.73a	62.56a	143.12a	169.19a	231.82a
D57xM06	37.19a	113.95ab	140.95a	259.59b	231.04a
58	47.46a	102.40ab	276.97b	351.13c	398.94b
56	129.79b	132.14b	319.90b	436.07d	417.97b
56x58	154.85b	213.29c	403.10c	559.00e	422.88b

[1] Means in columns followed by the same letter are not significantly different at the 5% level

L.S.D. 5%	Main effect	Interaction
Cultivars	37.38	121.49
Infestation level	49.76	67.34

At the 4.2 larval level, 56 and 56x58 had a significantly higher larval biomass/plant than had the other cultivars. As infestation levels increased, so the differences between cultivars became more significant, reaching the greatest difference for the 17.2 larval level. As seen with the numbers of larvae and larval mass at the 33.8 level, significant differences decreased between cultivars. At this level there was only a significant difference

between the D57/M06 and 56/58 groups, with no significant differences within each group.

Analysis of data for each cultivar across infestation levels revealed a significant difference for M06 only between the 33.8 levels and all other levels except the 17.2 level. For D57 there were significant differences between the 4.2 level and the 17.2 and 33.8 levels. For D57 x M06 there were significant differences between the low levels (4.2 and 8.9) and 17.2 and 33.8 levels, but no significant differences between the higher 3 levels (13.4, 17.2 and 33.8). Significant differences appear between 4.2 and the 13.4 level and higher for 58, 56 and 56 x 58. There were no significant differences between the 17.2 and 33.8 levels for all cultivars except for 56 x 58 which had a significantly higher value for the 17.2 level.

Analysis of Correlation (measure of linear association) showed the following relationship between the leaf damage ratings, numbers of larvae, larval mass and larval biomass:

Table 4.1.15. Correlation matrix of leaf damage ratings, numbers of larvae, larval mass and larval biomass

	1	2	3	4
1. Leaf damage	1			
2. Larval numbers	+0.54**	1		
3. Larval mass	-0.69**	-0.43**	1	
4. Larval biomass	+0.87**	+0.96**	-0.90**	1

5% r (88 D.F.) = 0.21*
1% r (88 D.F.) = 0.28**

There was a moderate but highly significant ($P<0.01$) positive correlation between leaf damage and numbers of larvae ($r = +0.54$) feeding in the cultivars 28 days after infestation. The rate of damage increase was curvilinear, reducing as numbers of larvae

increased. A highly significant ($P<0.01$) negative correlation ($r = -0.69$) occurred between leaf damage and larval mass, indicating a negative linear relationship between larval mass and numbers. This apparent anomaly can be explained by the decreasing mean mass/larva with increasing numbers of larvae ($r = -0.43$, $P<0.01$). Thus with increasing numbers of larvae, competition between larvae resulted in smaller larvae but an overall greater larval biomass, which was strongly correlated to leaf damage ($r = 0.87$, $P<0.01$).

Larval biomass was also strongly positively correlated with numbers of larvae ($r = +0.96$, $P<0.01$), and significantly negatively correlated with larval mass ($r = -0.90$, $P<0.01$) due to the negative correlation between numbers of larvae and larval mass.

Larval development and damage in maize is therefore a complex picture, influenced predominantly by the presence or absence of resistant factors in the maize cultivar, the number of larvae feeding, duration of that feeding period, the development or increase in mass of larvae, and the resultant competition between the larvae due to all these factors. As will be discussed later, the age of the plant and the plant tissue being consumed also markedly affect larval development, and therefore yield loss.

4.1.1.2 Larval survival and development in different maize genotypes

It was concluded in 4.1.1.1. that:

(a) Resistance mechanisms affecting numbers of larvae and larval mass were present in certain genotypes and were operative on larvae feeding in whorl tissue.

(b) One resistance mechanism resulted in fewer larvae surviving in resistant germplasm than in susceptible germplasm.

(c) Another resistance mechanism resulted in larvae

developing at a slower rate in resistant germplasm than larvae feeding in susceptible germplasm.

(d) The smaller larval biomass in the resistant cultivars resulted in less leaf feeding damage; there were strong correlations between leaf damage, numbers of larvae, larval mass and larval biomass.

(e) The resistance observed in D57 and M06, and the susceptibility observed in 56 and 58 were heritable traits. Damage caused in single crosses containing these lines was correlated with the resistance / susceptibility of the constituent inbred parents.

The objectives of the following experiment were to confirm the above conclusions, and to further investigate:

(a) The extent of leaf feeding damage caused by stalk borer larvae feeding in different maize genotypes (see 5.2.1).

(b) The effect of the different maize genotypes on the number, mass and biomass of larvae feeding in each genotype.

(i) Materials and methods

The eleven maize inbreds chosen for the experiment had previously been screened with many others for resistance to *B. fusca* larvae. Their selection was designed to give a wide range of leaf damage.

The experiment was planted on 4th November 1982 using a randomized complete block design, with split plots and 4 replications. Each inbred row (whole plot) contained 20 plants spaced 22,5 cm apart, giving a total of 80 plants for each inbred. The split plot treatments of 10 plants each were two sampling dates (15 days and 25 days larval feeding). All plants were infested thirty-one days post-emergence with approximately 21 larvae (mean of 20,8 larvae/plant over the whole trial) applied with a "bazooka" on 15th December 1982. Leaf damage was assessed after 21 days feeding, by visually rating the damage on each plant on a 1 to 5 scale. These results are reported in

5.2.1.

Of the 80 plants of each inbred, 40 were removed from the field after 15 days feeding, and the remaining 40 plants removed after 25 days feeding. The whorl of each plant was unrolled and the larvae feeding therein were counted and weighed.

(ii) Results and discussion

(a) Numbers of larvae

There was a highly significant ($P<0.01$) difference between the numbers of larvae recovered from each inbred. There was no significant difference between the numbers of larvae recovered at the two sampling dates. The interaction between the two sources of variation was highly significant ($P<0.01$). Table 4.1.16. summarizes the results of the Analysis of Variance.

Table 4.1.16. Significance of mean numbers of larvae/plant recovered from 11 inbreds, after 2 feeding periods, (15 and 25 days feeding)

SOURCE OF VARIATION	F	F distribution values	
		5%	1%
Inbreds	13.97 **	2.18	3.00
Feeding periods	9.23 N.S.	4.17	7.56
Feeding periods x inbreds	3.86 **	2.18	3.00

C.V.% Whole Plots = 17.6%
Sub-plots = 22.1%

The effect of inbreds on numbers of larvae

Table 4.1.17. Mean numbers of larvae/plant recovered from 11 inbreds, averaged over 2 feeding periods

INBRED										
F08	D57	D55	D50	F23	K11	F03	D53	56	M23	D54
2.23	2.27	2.61	2.86	3.45	3.71	3.75	4.01	4.27	4.27	4.71
a[1]	a	a	ab	bc	cd	cd	cd	de	de	e

[1] **Means followed by the same letter are not significantly different at the 5% level**

L.S.D. (5%) = 0.66

It is evident that F08, D57, D55 and D50 have an antibiotic resistance mechanism that reduced the number of larvae feeding in the whorl tissue. This contrasts markedly with the lines 56, M23 and D54, for example, which lack such a mechanism. The mechanism appears to be additive as there was a gradual increase in larval numbers over all inbreds.

The effect of feeding period on numbers of larvae

The mean numbers of larvae/plant removed from all inbreds after 15 and 25 days feeding (3.25 and 3.69 larvae/plant respectively (L.S.D. = 0.45)) were not significantly different. This is discussed more fully under the inbreds x days interaction.

The effect of the inbreds x feeding periods interaction on numbers of larvae/plant

The interaction between these two variates is shown in Table 4.1.18.

Table 4.1.18. An interaction table showing the mean number of larvae/plant recovered from 11 maize inbreds, after 15 and 25 days feeding

FEEDING PERIOD (DAYS)	F08	D57	D55	D50	F23	K11	F03	D53	S56	M23	D54
5	1.08a[1]	2.30b	2.05b	3.35c	3.30c	3.52c	3.37c	4.07cd	3.87cd	4.57d	3.47c
25	2.62l	2.25l	3.17lm	2.37l	3.60m	3.90mn	4.12mn	3.95mn	4.67n	3.97mn	5.95o
	*	N.S.	*	*	N.S.	*	N.S.	N.S.	N.S.	N.S.	*

[1]Means in rows followed by the same letter are not significantly different at the 5% level

L.S.D.(5%)	Main effect	Interaction
Inbreds	0.66	0.95
Feeding periods	0.34	0.96

There was a resistance mechanism present in some inbreds which reduced numbers of larvae within the first fifteen days of feeding. From an initial infestation of a mean of 20.8 larvae per plant, a large reduction in numbers of larvae occurred. The values ranged from a mean of 1.08 larvae/plant for F08, to the highest number of 4.57 larvae/plant for M23. Between the 15 and 25 days feeding there was no further significant change in numbers of larvae in D57, F23, F03, D53, S56 and M23. There were significant changes in larval numbers in the other five inbreds. It is also pertinent to point out that different plants to those sampled at the 15 day sampling, with different initial larval levels, were sampled at the 25 day period. This, coupled with migration, may explain the fact that some inbreds showed an increase in numbers of larvae (F08, D55, F23, K11, F03, 56, D54). The data indicate that the resistant factor affecting

numbers of larvae operated only within the first fifteen days of larval feeding.

(b) Larval mass

Table 4.1.19. summarizes the results of the Analysis of Variance, comparing the mean larval mass, for 11 inbreds, over 2 feeding periods:

Table 4.1.19. Significance of mean larval mass (mg) of larvae recovered from 11 inbreds after 2 feeding periods, 15 and 25 days feeding

SOURCE OF VARIATION	F	F distribution values	
		5%	1%
Inbreds	24.80 **	2.18	3.00
Feeding periods	414.60 **	4.17	7.56
Inbreds x Feeding periods	12.60 **	2.18	3.00

C.V.% Whole Plots = 12.4%
Sub-plots = 24.3%

There was a highly significant ($P<0.01$) difference between the mean larval mass of larvae recovered from each inbred, and also from each sampling date. The interaction between the two variates was also highly significant ($P<0.01$).

The effect of inbreds on larval mass

Table 4.1.20. Mean mass (mg) of larvae recovered from each of 11 inbreds, averaged over 2 feeding periods

INBREDS										
F08	D50	D57	D55	D53	56	K11	M23	F23	F03	D54
9.8	11.4	12.1	15.8	25.3	33.0	34.5	35.6	46.1	61.7	62.4
a[1]	a	a	ab	bc	c	c	cd	d	e	e

[1] **Means followed by the same letter are not significantly different at the 5% level**

L.S.D. (5%) = 11.1

F08, D50, D57 and D55 had a resistance mechanism that retarded larval development. Conversely, F03 and D54 did not possess such a mechanism. It can be seen that larvae feeding in these inbreds weighed several times as much as larvae feeding in F08, D50, D57 and D55. There appears to be less resistance in D53, 56, K11, M23 and F23, but the growth of larvae was nevertheless affected. As theorised with the resistance affecting numbers of larvae, an additive mechanism appears to be operative on larval mass, as a continuous range in values was recorded.

It is interesting to compare the possession of the two resistance mechanisms in the inbreds.

INBRED	NUMBERS OF LARVAE/PLANT	MEAN LARVAL MASS (mg)
F08	2.23 a[1]	9.8 a[1]
D50	2.86 ab	11.4 a
D57	2.27 a	12.1 a
D55	2.61 a	15.8 ab
D53	4.01 cd	25.3 bc
56	4.27 de	33.0 c
K11	3.71 cd	34.5 c
M23	4.27 de	35.6 cd
F23	3.45 bc	46.1 d
F03	3.75 cd	61.7 e
D54	4.71 e	62.4 e

[1]Means in columns followed by the same letter are not significantly different at the 5% level

With the exception of F23, inbreds that had fewer larvae also showed a resistance mechanism which retarded larval growth. It appears therefore, that the two mechanisms could be linked, but as will be shown later, these two mechanisms are nevertheless distinct and separate (see 4.1.1.3). If the same number of larvae were feeding in all genotypes, the differences in mass would probably be greater, as it was shown in 4.1.1.1. that the greater the number of larvae feeding, the lower the individual mass.

The effect of feeding period on larval mass

The mean mass of larvae removed from the plants after 15 and 25 days feeding were 8.0mg and 55.2mg per larva respectively. This difference was highly significant ($P<0.01$), with rapid mass gains being achieved during the period 15 to 25 days feeding (L.S.D. = 4.7).

The effect of the inbreds x feeding periods interaction on larval mass

The interaction between inbreds and feeding periods was highly significant ($P<0.01$).

Table 4.1.21. Mean larval mass (mg) of larvae recovered from 11 inbreds, after 15 and 25 days feeding

FEEDING PERIODS	INBREDS										
	F08	D50	D57	D55	D53	56	K11	M23	F23	D54	F03
15 Days	2.5a[1]	3.6a	2.7a	2.9a	5.8a	9.6a	7.1a	6.0a	9.4ab	15.7ab	22.2b
25 Days	16.5q	19.2q	21.5q	28.7q	44.8r	56.4rs	61.9s	65.2s	82.8t	109.2u	101.3u
	N.S.	*	*	*	*	*	*	*	*	*	*

L.S.D. 5%	Main effect	Interaction
Inbreds	11.7	15.7
Feeding periods	4.7	15.7

[1] Means in rows followed by the same letter are not significantly different at the 5% level

All inbreds except F08, showed significant differences between the mean larval mass from collections made after 15 and 25 days feeding. The susceptibility of F23, D54 and F03 was especially evident. Mass increases of 73.4mg, 93.5mg and 79.1mg, occurred respectively for these inbreds, compared with mass increases of only 14.0mg and 15.6mg for F08 and D50 respectively. It is possible that the resistance in F08, D50, D57 and D55 is longer lasting than that of D53, 56, K11, M23 and F23, as the mean larval mass increased at a greater rate in these latter inbreds. The intermediate levels of resistance initially shown by D53, 56, K11, M23 and F23 had certainly dissipated by the 25 day sampling.

There were few significant differences between inbreds at the 15 day sampling. Only larvae feeding in F03 showed a significantly ($P<0.05$) different mass to larvae sampled from all other inbreds

except F23 and D54. The differences at the 25 day collection were much greater with larvae from D54 weighing the most (109.2 mg) and larvae from F08 still weighing the least (16.5 mg). The relative rankings of the lines, however, did not change substantially. Whatever resistance mechanism was active at the 15 day sampling preventing larvae from utilizing plant tissue effectively, was still exerting its effect in F08, D50, D57 and D55 after 25 days feeding. The quantitative expression of several genes acting additively is clear. The resistance is more clearly illustrated by the increase in mass for larvae feeding in each inbred (Fig.4 and Table 4.1.22).

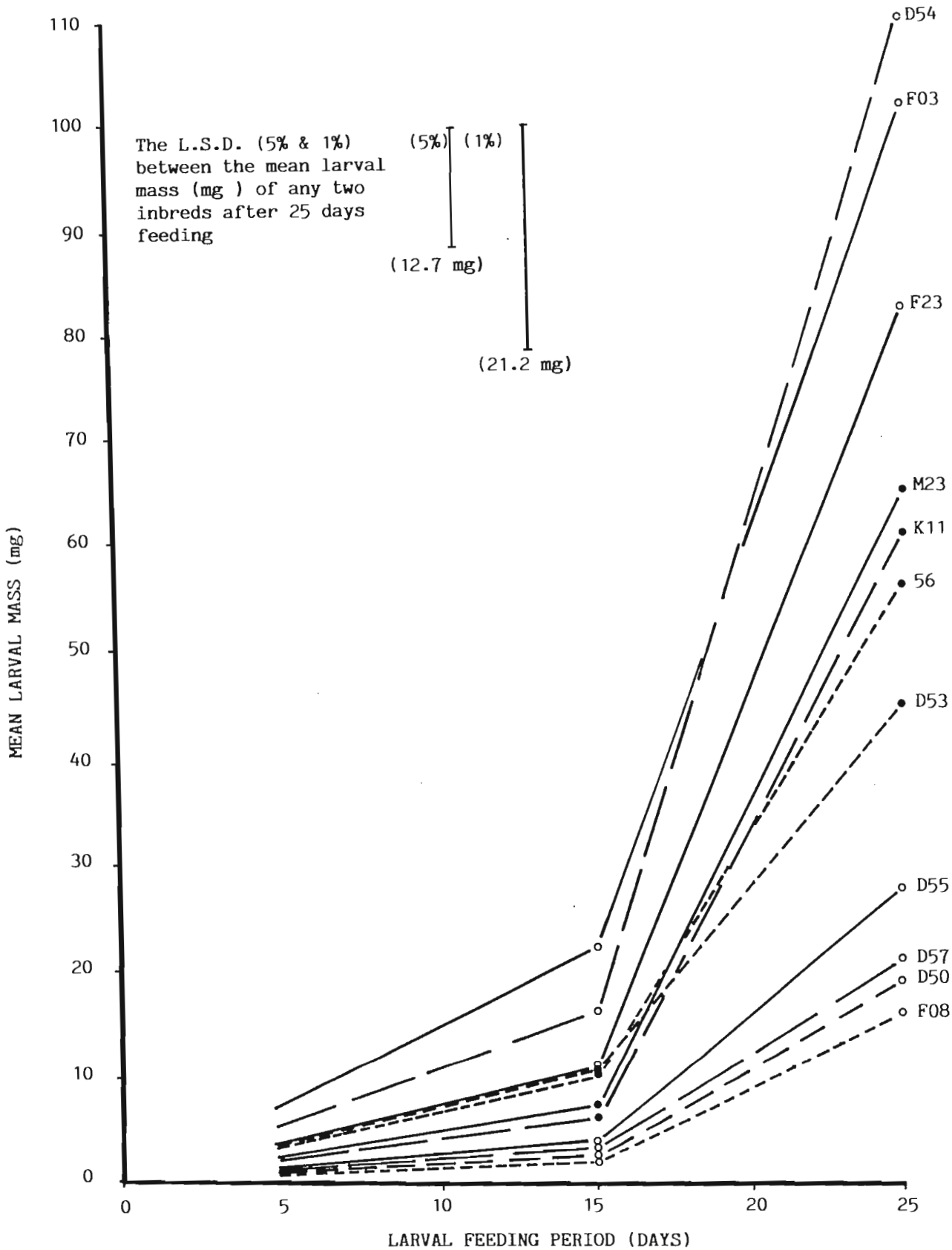


Fig. 4. Mean mass (mg) of B. fusca larvae feeding on 11 maize inbreds, removed after 15 and 25 days of feeding

Table 4.1.22. Increase in mean larval mass (mg) of larvae feeding for the 10 days between the 15 and 25 days feeding collections

INBREDS										
D50	D57	D55	D53	56	K11	M23	F23	F03	D54	F08
14.0	15.6	18.8	25.8	39.0	46.8	54.8	59.2	73.4	79.0	93.5

There was a rapid gain in larval mass of larvae feeding on certain of the maize inbreds (D54, F03 and F23) during the 10 day period after the 15 day sampling. In others (F08, D50, D57 and D55) there appeared to be a resistant factor retarding larval development. As larvae feed in leaf tissue for about thirty days, the resistance is long lasting and would be of benefit in the development of resistant germplasm.

(c) Larval biomass

The data indicate that two resistance mechanisms were active against stalk borer larvae feeding in the whorls in some maize genotypes. One mechanism reduced the number of larvae surviving in plants, and the other retarded their mass gain. Both phenomena, either acting singly or together resulted in a total biomass of larvae feeding. Table 4.1.23. summarizes the Analysis of Variance of the mean larval biomass for 11 inbreds.

Table 4.1.23. Significance of mean larval biomass recorded in 11 inbreds after 2 feeding periods, 15 and 25 days feeding

SOURCE OF VARIATION	F	F distribution values	
		5%	1%
Inbreds	52.60 **	2.18	3.00
Feeding periods	362.64 **	4.17	7.56
Inbreds x Feeding periods	22.52 **	2.18	3.00

C.V. % Whole Plots = 19.4%
Sub-plots = 21.9%

There was a highly significant ($P<0.01$) difference between the mean larval biomass recovered from each inbred, and also from each sampling date. The interaction between the two variates was also highly significant ($P<0.01$).

The effect of feeding period on larval biomass

The mean larval biomass removed from the plants after 15 and 25 days feeding were 26.8 mg and 228.3 mg per plant respectively. This difference was highly significant ($P<0.01$) (L.S.D. = 21.4).

The effect of inbreds on larval biomass

There were highly significant differences ($P<0.01$) between inbreds with regard to the mean larval biomass removed from all plants (Table 4.1.24.).

Table 4.1.24. Mean larval biomass/plant (mg) removed from each of 11 inbreds, averaged over 2 feeding periods

INBREDS										
F08	D57	D50	D55	D53	K11	M23	56	F23	F03	D54
24.6	29.1	29.2	48.7	100.5	114.7	147.8	149.2	162.9	246.1	350.6
a[1]	a	a	a	b	bc	cd	cd	d	e	f

[1] Means followed by the same letter are not significantly different at the 5% level

L.S.D. (5%) = 40.3

The inbreds F08, D57, D50 and D55 had a very low larval biomass (24.6 to 48.7 mg/plant). There was an intermediate group of D53, K11, M23, 56 and F23 (100.5 to 162.9 mg/plant), and two inbreds F03 and D54 which had the highest larval biomass/plant of 246.1mg & 350.6 mg/plant respectively.

The relative contributions of the two resistance mechanisms are tabulated below along with the resultant leaf damage(see 5.2.1.):

Table 4.1.24. Mean larval biomass/plant (mg) recovered from each of 11 inbreds, averaged over 2 feeding periods

INBREDS										
F08	D57	D50	D55	D53	K11	M23	56	F23	F03	D54
24.6	29.1	29.2	48.7	100.5	114.7	147.8	149.2	162.9	246.1	350.6
a[1]	a	a	a	b	bc	cd	cd	d	e	f

[1] Means followed by the same letter are not significantly different at the 5% level

L.S.D. (5%) = 40.3

The inbreds F08, D57, D50 and D55 had a very low larval biomass (24.6 to 48.7 mg/plant). There was an intermediate group of D53, K11, M23, 56 and F23 (100.5 to 162.9 mg/plant), and two inbreds F03 and D54 which had the highest larval biomass/plant of 246.1mg & 350.6 mg/plant respectively.

The relative contributions of the two resistance mechanisms are tabulated below along with the resultant leaf damage(see 5.2.1.):

Table 4.1.25. Ranking of inbreds according to the mean leaf damage ratings, numbers of larvae, larval mass and larval biomass per plant, averaged over both feeding periods

	RANKINGS ACCORDING TO:			
Inbred	Leaf Damage Ratings/Plant	Numbers of Larvae/plant	Mass of Larvae	Larval Biomass
F03	1 a[1]	7 cd	10 e	10 e
F23	2 ab	5 bc	9 d	9 d
D57	3 ab	2 a	3 a	2 a
D50	4 ab	4 ab	2 a	3 a
F08	5 bc	1 a	1 a	1 a
D55	6 bcd	3 a	4 ab	4 a
M23	7 de	9 de	8 cd	7 cd
D53	8 ef	8 cd	5 bc	5 b
D54	9 ef	11 e	11 e	11 f
K11	10 f	6 cd	7 c	6 bc
56	11 g	9 de	6 c	8 cd

1. Inbreds arranged in order of increasing leaf damage rating.

[1]Ranks in columns followed by the same letter are not significantly different at the 5% level

With the exception of F03 and F23, the rankings are similar across these relative attributes. A group of D57, D50, F08 and D55 can be classed together with low recordings of leaf damage, numbers of larvae, larval mass and larval biomass, and the other inbreds (M23, D53, D54, K11 and 56) can be classed in another group. The anomaly of F03 and F23 showing low leaf damage but high numbers, mass, and biomass of larvae is discussed overleaf.

What is of practical significance is the good correlation between larval development and leaf damage. Leaf damage is a quick method of assessing plant damage, and in this table can be seen to represent the effect of plant resistance on larvae in 80% of the inbreds screened. Although F03 and F23 had larger larvae, the low damage is what is required in maize plants, irrespective of what is happening to the larvae.

The effect of inbreds x days interaction on larval biomass

Table 4.1.26. Mean larval biomass/plant (mg) of larvae r recovered from 11 inbreds, after 15 and 25 days feeding

	INBREDS										
FEED. PERIOD (DAYS)	F08	D57	D50	D55	D53	K11	M23	56	F23	F03	D54
15	4.6a[1]	6.2a	11.8a	5.8a	23.4a	25.8a	25.5a	36.6a	30.3a	71.9b	54.9a
25	44.6l	51.9l	46.6l	91.5l	177.6m	203.1mn	270.1no	261.7no	295.4o	420.4p	648.2q
	N.S.	N.S.	N.S.	*	*	*	*	*	*	*	*

L.S.D. 5%	Main effect	Interaction
Inbreds	40.3	71.6
Feeding periods	21.5	64.7

[1] Means in rows followed by the same letter are not significantly different at the 5% level

At the 15 day sampling, although there were large differences, only F03 showed significantly heavier larvae than any other inbred. By the 25 day sampling, the differences between inbreds were large and significant ($P<0.05$). F08 still had the lowest biomass (44.6mg) and D54 had the heaviest biomass (648,2mg). The

fact that D54 had a lower biomass (54.9mg) than F03 (71.9mg) at the 15 day sampling, but a higher biomass (648.2mg) than F03 (420.4mg) at the 25 day sampling, could indicate some late acting resistant factor in F03.

All inbreds except F08, D57 and D50 showed significant ($P<0.05$) larval biomass gains during the 10 day period between 15 and 25 days. These data confirm that these three lines evidently have a long lasting resistant mechanism retarding larval mass gain.

As was seen with the data on larval mass, the differences between inbreds increased with prolonged feeding time. The increases in biomass are shown in Table 4.1.27. and Fig.5.

Table 4.1.27. Increase in mean biomass/plant (mg) of larvae feeding for the 10 days between the 15 and 25 days feeding samplings

INBREDS										
D50	F08	D57	D55	D53	K11	58	M23	F23	F03	D54
34.8	40.0	45.7	85.7	154.2	177.8	225.1	244.6	265.1	348.5	595.3

Two inbreds show anomalous reactions in Figure 5. The inbreds F03 and F23 show a surprisingly high larval biomass, but the lowest leaf damage (Table 4.1.25). It is surmised that the nutritional status of these two inbreds is high, allowing larvae to consume the same amount of whorl tissue as larvae feeding in other resistant inbreds (D57, D50 and F08 for example), yet to gain mass far quicker. If these two inbreds are excluded from a correlation analysis between mean larval biomass/plant after 15 days feeding and leaf damage ratings after 21 days feeding, a highly significant positive correlation occurs ($r = +0.65$, $P<0.01$) between these data.

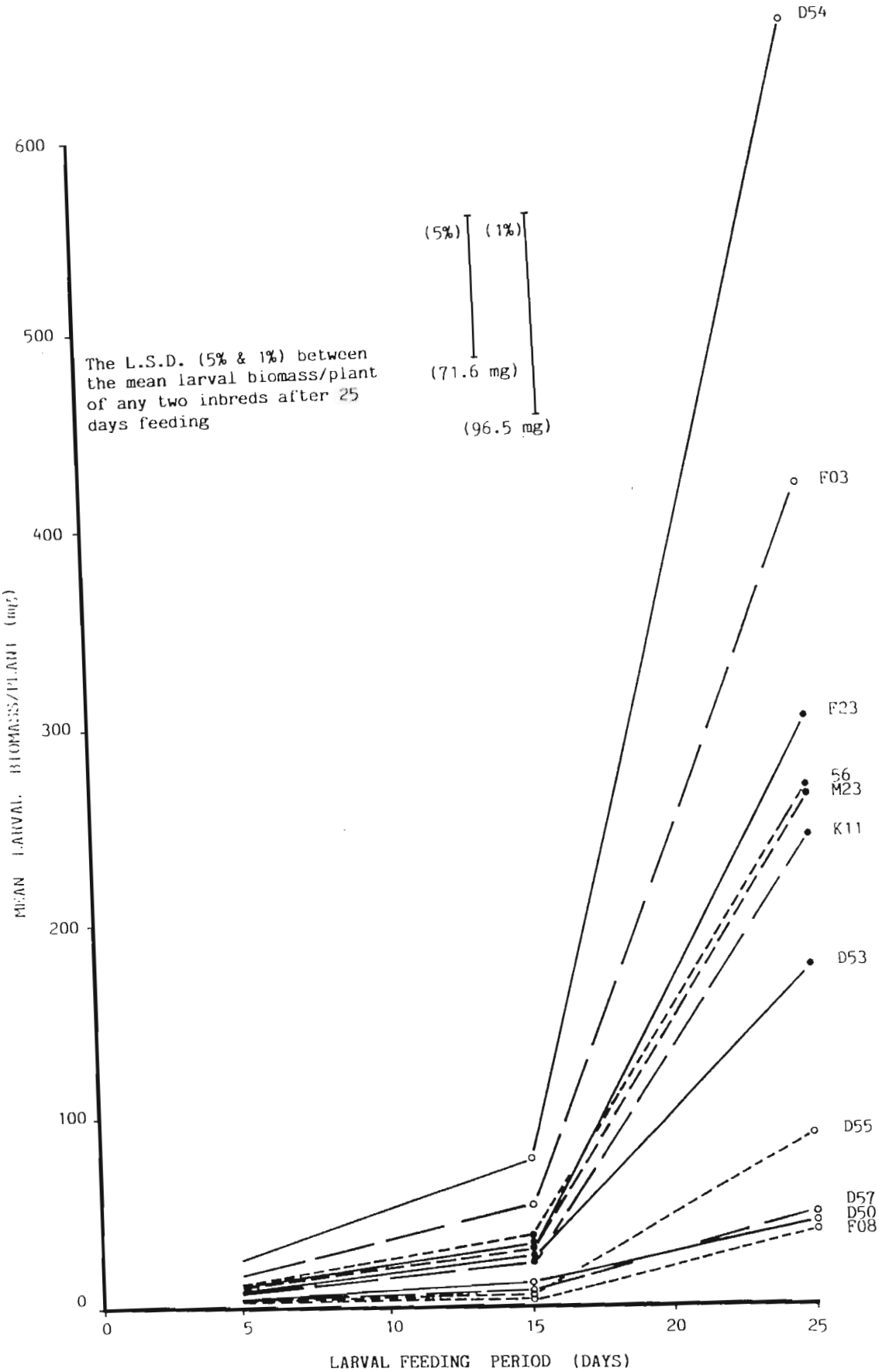


Fig. 5. Mean larval biomass/plant (mg) of B. fusca larvae removed from 11 maize inbreds, after 15 and 25 days of feeding

Plants having a low nutritional status can be considered as having resistance to stalk borer larvae. However this resistance may not necessarily be due to the occurrence of an antibiotic chemical, as is the case with the European Corn Borer (*Ostrinia nubilalis*). Robinson *et al.* (1982) showed that this resistance is due to the chemical 2,4 - dihydroxy - methoxy - 1,4 - benzoxazin-one which affects larval development. A high nutritional status (as opposed to a plant having a resistant factor) will result in rapid larval growth and very little leaf damage. The result is the same as a plant having a resistant factor which reduces larval growth and hence limits damage. The nutritional status would possibly have a minimal effect on numbers of larvae. This hypothesis is shown in Table 4.1.28, where an attempt is made to describe the reasons for the different larval and plant responses.

Any one type of plant response can be the result of any one of eight different interactions between resistant mechanisms affecting numbers of larvae, larval mass (and hence biomass) and the nutritional status of the plant.

Table 4.1.28: Summary of hypothetical interaction between resistance affecting larval numbers, resistance affecting larval mass gain, the nutritional status of the maize genotypes, and the effect on stalk borer and plant response

RESIST. TO NUMBERS	RESIST TO MASS GAIN	NUTRITIONAL STATUS (LEAF)	PROBABLE LARVAL RESPONSE		POSSIBLE REASON FOR PLANT RESPONSE	
			NUMBERS	MASS GAIN	LOW PLANT DAMAGE	HIGH PLANT DAMAGE
YES	YES	LOW	LOW	LOW	1. Few larvae of low mass eating non-nutritious food: strongest expression of plant resistance to stalk borer.	2. Would not occur.
YES	NO	LOW	LOW	LOW	3. Few larvae of low mass eating non-nutritious food resulting in slow mass gain.	4. Few larvae of low mass, having to consume vast quantities of food in order to survive. Resistance ineffective in preventing plant damage.
YES	YES	HIGH	LOW	LOW	5. Few larvae of low mass unable to utilize high nutritional value of food.	6. Few larvae of low mass potential able to increase mass through high nutritional status of food. Resistance ineffective.
YES	NO	HIGH	LOW	HIGH	7. Few larvae, despite high mass, unable to cause extensive damage due to low numbers	8. Few larvae their maturing due to nutritious food, consuming plant parts quicker than the plant could grow. Would probably not be a common phenomenon.
NO	YES	LOW	HIGH	LOW	9. Many, but very small larvae eating non-nutritious food.	10. Many larvae o f low mass consuming large amounts of food without being able to utilize it.
NO	YES	HIGH	HIGH	LOW	11. Many larvae of low mass unable to capitalise on high nutritional state of food.	12. Many small larvae eating small amounts of highly nutritious food. Not a common phenomenon
NO	NO	LOW	HIGH	LOW	13. Many larvae unable to gain mass due to low nutritional food.	14. Many larvae consuming large amounts of food without being able to utilize it.
NO	NO	HIGH	HIGH	HIGH	15. Would not occur.	16. Many large larvae causing extensive damage. Extreme susceptibility.

Plant damage is obviously not just a simple response to one or more resistant factors affecting larval populations or growth. The general nutritional status of the plant, in its broadest terms, is an integral partner in the complex picture of insect-plant interaction. The simple objective of any HPR programme is the reduction of damage to a sub-threshold level. It is of no importance if that objective is achieved by breeding plants that have resistant factors which limit the larval population or larval growth, or whether it is achieved by developing plants that have such a high nutritional status that the insect can complete its life cycle in a short enough time to cause minimal damage.

4.1.1.3: Larval survival and development in different maize hybrids

This experiment was planned to complement 4.1.1.2, by screening various single cross hybrids which contained several inbreds from 4.1.1.2. However, due to insufficient seed quantities, new unscreened inbreds were used as parents of some of the hybrids to make up a trial of reasonable size. The major objective was to assess the heritability of resistance. Could one predict the effect on larvae of single cross hybrids, based on the individual effects of each constituent parent? In addition it was hoped that light would be shed on the linkage between the two resistance mechanisms that affected larval numbers and mass gain.

(i) Materials and methods

The layout, planting date and planting methods were as described in 4.1.1.2. The whole plot treatments (2 rows of 10 plants) were two feeding periods (15 and 25 days feeding), and the sub-plot treatments were the 10 single cross hybrids. All plants were infested 35 days post-emergence with a mean of 19.6 neonate larvae applied with a "bazooka" to each plant. The effect of the hybrids on larval development was assessed by cutting open half the number of plants after 15 days feeding, and the remaining plants after 25 days feeding, and counting and weighing the larvae.

(ii) Results and discussion

(a) Numbers of larvae

There was a highly significant ($P<0.01$) difference between the numbers of larvae recovered from each hybrid. There was no significant difference in the numbers of larvae recovered at the two sampling dates. The hybrids x feeding period interaction was also not significant.

Table 4.1.29. summarizes these results:

Table 4.1.29. Significance of mean numbers of larvae/plant recovered from 10 single cross hybrids, after 2 feeding periods, 15 and 25 days feeding

SOURCE OF VARIATION	F	F distribution values	
		5%	1%
Hybrids	25.29 **	2.30	3.29
Feeding periods	0.37 N.S.	4.17	7.56
Hybrids x feeding periods	1.76 N.S.	2.27	3.17

C.V. % Whole Plots = 10.0%
Sub-plots = 21.3%

The effect of hybrids on larval numbers

There were highly significant ($P<0.01$) differences between hybrids with regard to the numbers of larvae removed from all plants (Table 4.1.30). This was probably due to the occurrence of a resistance mechanism, which reduced numbers of larvae, in those hybrids with low larval counts.

Table 4.1.30. Mean numbers of larvae/plant recovered from all hybrids averaged over the two feeding periods

HYBRID	LARVAE/PLANT
D57 x F08	2.15 a[1]
D54 x F07	2.81 b
F08 x M23	2.84 b
M06 x J14	3.60 c
F08 x F70	3.64 c
F70 x D54	3.77 c
F23 x F03	4.61 d
56 x 58	4.61 d
D55 x D53	4.72 d
D53 x D50	4.94 d

[1] Means followed by the same letter are not significantly different at the 5% level

L.S.D. (5%) = 0.55

There were 3 broad groups:

(i) D57xF08; D54xF07; F08xM23 (showing the existence of a resistance mechanism which reduced numbers of larvae)

(ii) M06xJ14; F08xF70; F70xD54 (intermediate reaction),

(iii) F23xF03; 56x58; D55xD53; D53xD50 (showing a more susceptible reaction).

Because all the inbreds in 4.1.1.2. were not combined in the form of a diallel (a series of hybrids containing inbreds in all possible single cross combinations), it is not possible to speculate as to the relative contributions of each parent to the resistance of the hybrids. Where inbred data was available (see Table 4.1.17.), it was possible to comment on the parental and hybrid values.

Table 4.1.31. Mean numbers of larvae/plant recovered from 5 single cross hybrids and 10 inbreds (data from Table 4.1.17. common parents of the single cross hybrids), after 2 feeding periods (15 and 25 days feeding)

INBRED PARENTS	LARVAE/PLANT *per se*	MEAN[2.]	HYBRID	LARVAE/ PLANT
D57	2.27 a[1.]	2.25	D57 x F08	2.15 l
F08	2.23 a			
F08	2.23 a	3.25	F08 x M23	2.84 m
M23	4.27 de			
F23	3.45 bc	3.60	F23 x F03	4.61 o
F03	3.75 cd			
D55	2.61 a	3.31	D55 x D53	4.72 o
D53	4.01 cd			
D50	2.86 ab	3.43	D50 x D53	4.94 o
D53	4.01 cd			

[1.] Means in each column followed by the same letter are not significantly different at the 5% level
[2.] Theoretical mean of both inbred parent values.

The most resistant single cross hybrid D57 x F08 also had the most resistant parents, which would appear consistent so far with an additive mode of resistance. But when two similar groups of parents were compared, conclusions are not so easily reached:

The theoretical mean of 3.25 larvae/plant was calculated for F08 (2.23) and M23 (4.27). A similar mean of 3.31 larvae/plant was obtained for D55 (2.61) and D53 (4.01). However, their single cross hybrids differed greatly: F08 x M23 = 2.84 larvae/plant

and D55 x D53 = 4.72 larvae/plant.

Predictive value of data gained from inbreds was therefore low in forecasting which hybrid would have the least larvae.

There was a mean of 3.47 larvae/plant found in the inbreds (small plants) in 4.1.1.2., and a mean of 3.76 larvae/plant found in these single cross hybrids (large plants). This indicated that plant size was unimportant in larval establishment.

The effect of feeding period on numbers of larvae

There was no significant difference between the mean numbers of larvae/plant removed from the hybrids after 15 days (3,71 larvae/plant) and 25 days (3,82 larvae/plant) (L.S.D. = 2.28). These figures compare well with 3.25 larvae/plant and 3.69 larvae/plant removed after similar feeding periods in 4.1.1.2.

The effect of the hybrid x feeding period interaction on numbers of larvae

Table 4.1.32. Mean numbers of larvae/plant recovered from 10 single cross hybrids, after 15 and 25 days feeding

HYBRID	FEEDING PERIODS		
	15 Days	25 Days	
57 x F08	1.97 a[1.]	2.32 l	N.S.
D54 x F07	2.87 ab	2.70 l	N.S.
F08 x M23	3.07 bc	2.60 l	N.S.
M06 x J14	3.35 bc	3.85 mn	N.S.
F08 x F70	4.00 cde	3.27 lm	N.S.
F70 x D54	3.72 bcd	3.82 mn	N.S.
F23 x F03	4.92 e	4.30 no	N.S.
56 x 58	4.47 de	4.75 nop	N.S.
D55 x D53	3.77 bcd	5.67 p	*
D53 x D50	4.97 e	4.90 op	N.S.
	3.71	3.82	N.S.

[1.] Means in columns followed by the same letter are not significantly different at the 5% level

L.S.D. 5%	Main effect	Interaction
Hybrids	0.55	1.16
Feeding periods	2.28	0.99

Only D55 x D53 showed a significant increase in numbers of larvae/plant over time. These larvae may have migrated out of

plants of other hybrids into the plants of this hybrid. It is also possible that plants of this hybrid in the second sub-plot may have had a higher initial infestation. It is interesting that D55 was one of three inbreds in 4.1.1.2. which showed a significant increase in larval numbers over the two feeding periods.

Comparisons between hybrids showed highly significant differences between numbers of larvae removed at each sampling date.

As it is the 25 day feeding data that is more closely related to the damage caused by borers feeding in maize, the figures from that column have the most relevance in the study on resistance.

The lowest number of larvae were extracted from D57 x F08, F08 x M23 and D54 x F07, which show varying degrees of resistance. The most larvae were removed from F23 x F03, 56 x 58, D55 x D53, and D53 x D50, all which show greater susceptibility.

(b) Larval mass

A summary is given below of the results of the Analysis of Variance, comparing the mean larval mass for 10 single cross hybrids, over 2 feeding periods.

Table 4.1.33. Significance of mean larval mass recovered from 10 single cross hybrids, after 2 feeding periods, 15 and 25 days feeding

SOURCE OF VARIATION	F	F distribution values	
		5%	1%
Hybrids	6.02 **	2.25	3.20
Feeding periods	665.79 **	4.17	7.56
Hybrids x Feeding periods	4.13 **	2.20	3.10

C.V.% Whole Plots = 19.0%
Sub-plots. = 21.4%

There was a highly significant (P<0.01) difference between the mean larval mass recovered from each hybrid, and also from each sampling date. The interaction between the two was also highly significant.

The effect of hybrids on larval mass

There were highly significant (P<0.01) differences between the mean larval mass of larvae removed from the hybrids.

Table 4.1.34. **Mean larval mass (mg) of larvae recovered from each of 10 single cross hybrids, averaged over 2 feeding periods**

HYBRID	MEAN MASS (mg)
D57 x F08	13.93 a[1]
M06 x J14	24.73 b
D55 x D53	26.84 bc
F08 x F70	27.62 bc
D54 x F07	28.27 bcd
F23 x F03	29.37 bcd
56 x 58	29.51 bcde
F70 x D54	34.61 cde
D53 x D50	36.03 de
F08 x M23	37.74 e

[1] **Means followed by the same letter are not significantly different at the 5% level**

L.S.D. (5%) = 7.79

Again, a range of values indicates an additive resistance mechanism affecting mass gain. These significant differences indicate that some hybrids like D57 x F08 had a resistance mechanism that retards larval mass gain. Obviously hybrids like D53 x D50 and F08 x M23 did not contain this resistance. This is

more fully discussed under the Hybrids x Feeding period interaction. The relative contributions that the resistance of the parent inbreds make to the resistance of the single cross hybrids are shown in Table 4.1.35.

Table 4.1.35. Mean larval mass (mg) of larvae removed from each of 5 single cross hybrids and 10 inbreds (common parents of the single cross hybrids), after 2 feeding periods (15 and 25 days feeding)

INBRED PARENTS	MEAN LARVAL MASS		HYBRID	MEAN LARVAL MASS (mg)
	per se	Mean[2.]		
D57	12.1 a[1.]	10.95	D57 x F08	13.93 l
F08	9.8 a			
D55	15.8 ab	20.55	D55 x D53	26.84 mn
D53	25.3 bc			
F23	46.1 d	53.90	F23 x F03	29.37 mno
F03	61.7 e			
D53	25.3 bc	18.35	D53 x D50	36.03 op
D50	11.4 a			
F08	9.8 a	22.70	F08 x M23	37.74 p
M23	35.6 cd			

[1.] Means in each column followed by the same letter are not significantly different at the 5% level

[2.] Theoretical mean of both inbred parents

Predictive value of inbred data for determining mean larval mass of hybrids was negligible. However it is interesting to note

that the hybrid D57 x F08 had the lowest mean larval mass/plant, and the two constituent parents also had the lowest mean larval mass. The single cross F23 x F03 should have the heaviest larvae, assuming additive action of the susceptibility of the parents, but does in fact show an intermediate mean larval mass.

The effect of feeding period on larval mass

The mean larval mass of larvae removed from the plants after 15 and 25 days feeding were respectively 7.36mg and 50.37mg per larva. The difference between these values is significant (L.S.D. = 21.18). This compares closely with figures of 8.0mg and 55.2mg per larva for larvae removed from the 11 inbreds in 4.1.1.2. Although larvae fed for 3 more days in the inbreds (which are smaller plants), it is interesting to note that some of the inbreds had larger larvae than were recovered from the hybrids.

As with the inbred data in 4.1.1.2, there was no significant correlation ($r = -0.17$, N.S.) between the mean larval mass/plant after 15 days feeding, and the resultant leaf damage appearing 6 days later. This was due to no significant differences occurring between mean larval mass of the hybrids after 15 days feeding (Table 4.1.36.).

The largest mean larval mass found after 25 days feeding in the hybrids was 64.71mg (D53 x D50), while the inbreds F23, F03 and D54 all had heavier larvae (82.8mg; 101.3mg; 109.2mg respectively), even considering that larvae in the inbreds fed for 3 extra days. This indicates no additional nutritional or other benefit inherent in the larger plants of the single cross hybrids.

The effect of hybrid x feeding period interaction on larval mass

Table 4.1.36. Mean larval mass (mg) of larvae recovered from 10 single cross hybrids, after 15 and 25 days feeding

HYBRID	MEAN LARVAL MASS (mg)	
	15 DAYS	25 DAYS
D57 x F08	3.38 a[1.]	24.49 l *
M06 x J14	6.49 a	42.97 m *
D55 x D53	5.40 a	48.27 m *
F08 x F70	4.41 a	50.82 m *
D54 x F07	6.77 a	49.77 mn*
F23 x F03	13.15 a	45.60 mn**
56 x 58	6.57 a	52.45 mn**
F70 x D54	8.77 a	60.45 no**
D53 x D50	7.34 a	64.71 o **
F08 x M23	11.28 a	64.20 o **

[1.] Means followed by the same letter are not significantly different at the 5% level

L.S.D. 5%	Main effect	Interaction
Hybrids	7.79	10.75
Feeding periods	21.18	10.99

All hybrids showed a significant increase in mean larval mass from 15 to 25 days feeding. Differences between hybrids were not significant after 15 days feeding; the smallest larvae were removed from D57 x F08 (3.38 mg/larvae) and the heaviest larvae were removed from F23 x F03 (13.15), but the difference of 9.77 mg was not significant.

However by the 25 day feeding sample, the mean larval mass in D57 x F08 (24.49mg) was significantly different ($P<0.05$) from the mean mass of larvae removed from all other hybrids. The difference between the lowest and highest mean larval mass recovered from the hybrids was not as large as the difference recorded from the inbreds in 4.1.1.2. The mean larval mass for the smaller inbreds ranged from 16.5mg (F08) to 109.2mg (D54) (a difference of 92.7mg), while those for the single cross hybrids ranged from 24.49mg (D57 x F08) to 64.71mg (D53 x D50) (40.2mg). The effect of size of plant on larval development is therefore not an important consideration in plant resistance breeding.

The hybrid D57 x F08 had the lowest number of larvae/plant and also had the lowest mean larval mass. This indicates the possession of both resistance mechanisms, which separately affect numbers and mass gain of larvae feeding in whorl tissue. Similarly, D53 x D50 showed no evidence of either resistance mechanism, as this hybrid had the second highest number of larvae per plant of all hybrids, as well as the heaviest larvae.

The hybrids F23 x F03 and D55 x D53 had the third and forth lowest larval mass respectively after 25 days feeding (Table 4.1.36) but ranked seventh and ninth respectively with regard to highest larval numbers (Table 4.1.32). This indicates that both had some resistance controlling larval mass gain, but no resistance controlling numbers of larvae. Conversely, F08 x M23 had the second heaviest larvae (susceptible reaction) but had the third lowest number of larvae (resistant reaction). The two resistance mechanisms are therefore not linked.

The mass gain shown by larvae feeding during the 10 day period between the 15 and 25 days data is shown in Fig. 6.

D57xF08 had a resistance mechanism which retarded larval mass gain. There is an intermediate group of 6 hybrids which showed slight resistance controlling larval mass gain, and a group of

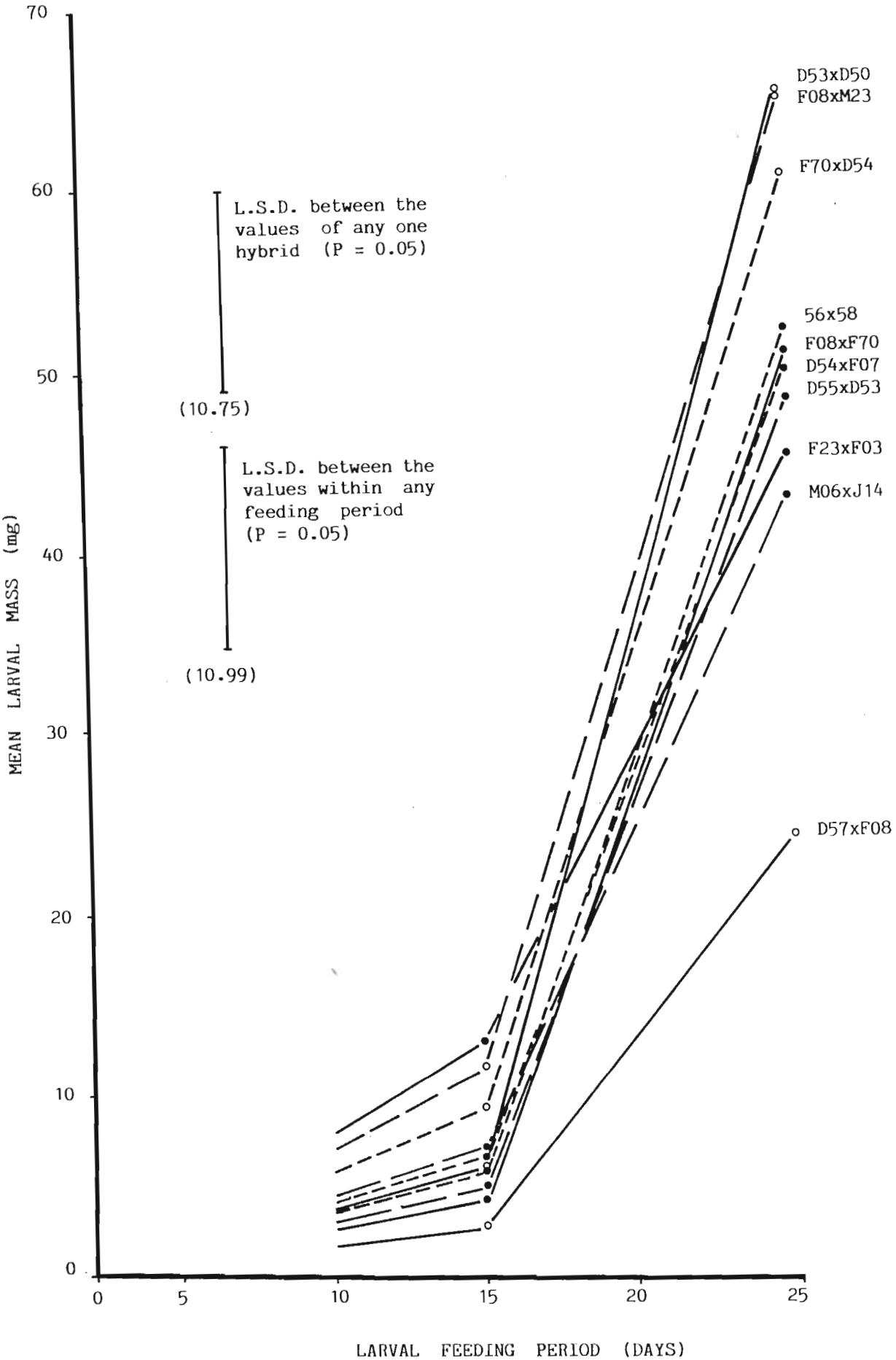


Fig.6 Mean larval mass increase of larvae feeding in 10 single cross hybrids for 25 days

3 hybrids which obviously did not have any such resistance mechanism.

Unlike the highly significant correlation found between numbers of larvae and larval mass in the inbreds of 4.1.1.2., there was no correlation between numbers of larvae and larval mass in these 10 hybrids (r = +0.08, N.S.).

(c) Larval biomass

Table 4.1.37 summarizes the results of the Analysis of Variance, comparing the mean larval biomass/plant recorded in each hybrid.

Table 4.1.37. Significance of mean larval biomass/plant in 10 single cross hybrids after 2 feeding periods, 15 and 25 days feeding

SOURCE OF VARIATION	F	F distribution values	
		5%	1%
Hybrids	20.06 **	2.25	3.20
Feeding periods	464.47 **	4.17	7.56
Hybrids x feeding periods	8.18 **	2.20	3.10

C.V. % Whole Plots = 16.4%
Sub-plots = 19.6%

There was a highly significant ($P<0.01$) difference between the mean larval biomass/plant recovered from each hybrid, and also from each sampling date. The interaction between the two variates was also highly significant.

<u>The effect of hybrids on larval biomass</u>

There were highly significant differences between hybrids with regard to the mean larval biomass/plant removed from all hybrids:

Table 4.1.38. Mean larval biomass (mg/plant) recovered from each of 10 hybrids, averaged over 2 feeding periods

HYBRIDS	LARVAL BIOMASS/PLANT (mg)
D57 x F08	32.3 a[1]
D54 x F07	77.1 b
F08 x F70	91.3 b
M06 x J14	93.6 b
F08 x M23	96.5 b
F23 x F03	126.6 c
F70 x D54	131.4 c
56 x 58	138.2 c
D55 x D53	146.4 c
D53 x D50	175.1 d

[1] Means followed by the same letter are not significantly different at the 5% level

L.S.D. (5%) = 26.4

The hybrid D57 x F08, having both resistance mechanisms, had the lowest biomass. The hybrid D53 x D50, lacking both resistance mechanisms, had the greatest biomass of all hybrids. The rankings of the other 8 hybrids correlate well with the rankings on larval numbers shown in Table 4.1.32. The first 5 hybrids in Table 4.1.38 which show the lowest biomass, are the same top ranking hybrids in Table 4.1.32., and the other 5 hybrids occur in the last 5 positions in both tables. It is interesting to tabulate the relative importance of the two resistance mechanisms controlling numbers and mass gain of larvae, and the resultant leaf damage:

Table 4.1.39. Ranking of hybrids according to mean leaf damage ratings, numbers of larvae, larval mass and larval biomass per plant, averaged over both feeding periods

	RANKINGS[1] ACCORDING TO:			
HYBRID	LEAF DAMAGE[2] RATINGS/PLANT	NUMBERS OF[3] LARVAE/PLANT	MEAN LARVAL[4] MASS	LARVAL[5] BIOMASS
F23 x F03	1 a	7 d	6 bcd	6 c
D54 x F07	2 a	2 b	5 bcd	2 b
D57 x F08	3 ab	1 a	1 a	1 a
F70 x D54	4 ab	6 c	8 cde	7 c
F08 x M23	5 b	3 b	10 e	5 b
F08 x F70	6 b	5 c	4 bc	3 b
M06 x J14	7 c	4 c	2 b	4 b
D55 x D53	8 c	9 d	3 bc	9 c
56 x 58	9 d	8 d	7 bcde	8 c
D53 x D50	10 d	10 d	9 de	10 d

1. Ranks in columns followed by the same letter are not significantly different at the 5% level. These rankings are based on the mean values attained from the relevant tables.
2. Table 5.27
3. Table 4.1.30
4. Table 4.1.34
5. Table 4.1.38

The hybrid F23 x F03, although showing the least leaf damage, ranked 7th, 6th and 6th for numbers of larvae, mass, and biomass respectively. Both inbreds in this hybrid were seen in 4.1.1.2. to also exhibit low leaf damage (F03 ranked 1st and F23 ranked 2nd), yet ranked 10th and 9th respectively with regard to larval

biomass. It was surmised that this anomaly was due to a very high nutritional status of these two inbreds, and it appears that this attribute has been carried through to the single cross hybrid.

The 3 worst ranked hybrids with regard to leaf damage (D55 x D53; 56 x 58; D53 x D50) were also ranked as the last 3 with regard to numbers of larval/plant and larval biomass. However, with the other hybrids, it is impossible to comment until more is known about the inheritance of the resistance mechanisms, and of the nutritional status of each inbred.

Correlation between larval biomass at 15 days and leaf damage ratings after 21 days feeding was not significant ($r = +0.12$, N.S.). The lack of correlation is probably due to interaction between the nutritional status of the plants and the resistance mechanisms present in the hybrids. If the data from the hybrid F23 x F03 is removed, correlation between biomass and leaf damage is significant ($r = +0.42$, $P<0.05$), as it was in 4.1.1.2. when data from inbreds were compared.

The effect of feeding period on larval biomass

The mean larval biomass/plant removed after 15 and 25 days feeding were 27.5 mg/plant and 194.2 mg/plant respectively. The difference between these values was found to be significant (L.S.D. = 98.2).

The effect of hybrid x feeding period on larval biomass

Table 4.1.40. Mean biomass of larvae recovered from 10 single cross hybrids, after 15 and 25 days feeding

HYBRID	MEAN LARVAL BIOMASS / PLANT (mg)	
	15 DAYS	25 DAYS
D57 x F08	6.7 m[1.]	57.9 a *
D54 x F07	18.2 m	136.0 b *
F08 x F70	17.9 m	164.6 bc *
M06 x J14	19.9 mn	167.3 bc *
F08 x M23	34.2 mn	158.7 bc *
F23 x F03	62.9 n	190.3 cd *
F70 x D54	32.7 mn	230.1 de *
56 x 58	28.3 mn	248.1 e *
D55 x D53	19.4 mn	273.5 ef *
D53 x D50	35.1 mn	315.0 f *

[1.] Means in columns followed by the same letter are not significantly different at the 5% level

L.S.D. 5%	Main effect	Interaction
Hybrids	26.42	43.9
Feeding period	98.21	49.8

At the 15 day sampling, only the values for D57 x F08 (lowest biomass), D54 x F07 and F08 x F70 were significantly ($P<0.05$) different from F23 x F03 (highest biomass). All other biomass values were not significantly different. By the 25 day sampling, there were large significant differences evident between hybrids: D57 x F08 still had the lowest larval biomass, and D53

x D50 had the highest (see Fig. 7).

All hybrids showed significant ($P<0.01$) biomass increases over time. Of great significance is the fact that in 4.1.1.2., out of 11 inbreds, F08 showed a non significant increase in biomass, and D57 actually showed a reduction in biomass. All other inbreds (except D50 = N.S.) showed highly significant ($P<0.01$) biomass gains. It is evident that the resistance shown in D57 and F08 is inherited in the single cross hybrid.

In Table 4.1.26, D50 showed a non significant biomass increase (and thus good resistance). However, in combination with D53 (intermediate reaction in 4.1.1.2.) the hybrid D50 x D53 showed the highest biomass gain, and thus the highest susceptibility, illustrating that the resistance in D50 is certainly not dominant.

The relative contributions that the resistance of the parent inbreds make to the resistance of the single cross hybrids is shown in Table 4.1.41.

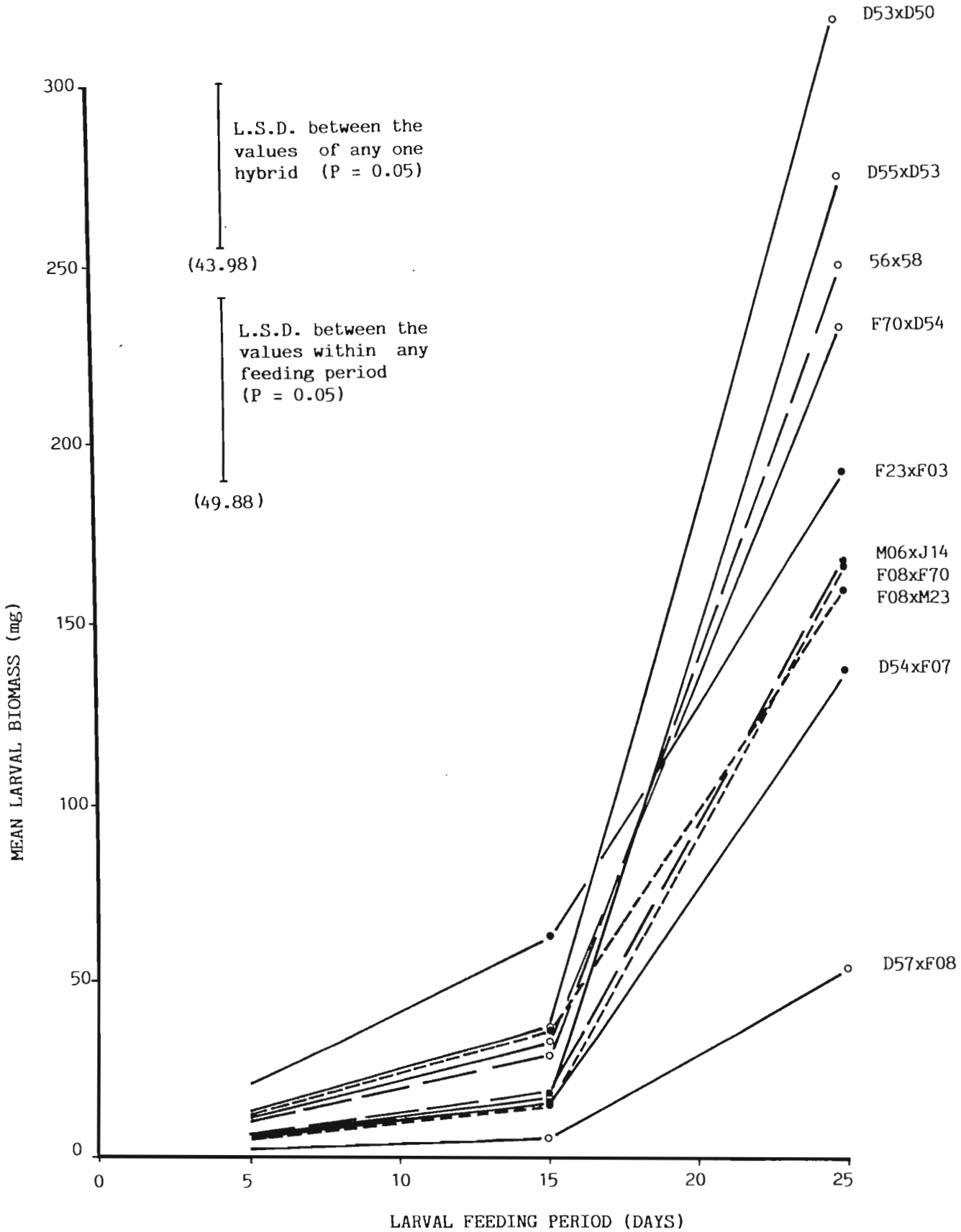


Fig.7. Mean larval biomass/plant increase of larvae feeding in 10 single cross hybrids for 25 days

Table 4.1.41. Mean biomass of larvae recovered after 25 days feeding from each of 5 single cross hybrids and 10 inbreds (common parents of the single cross hybrids, data from Table 4.1.26.)

INBRED PARENTS	LARVAL BIOMASS *per se*	LARVAL BIOMASS MEAN	HYBRID	MEAN LARVAL BIOMASS/PLANT
D57	51.9 l[1.]	48.2 [2.]	D57 x F08	57.9 a
F08	44.6 l			
D55	91.5 l	134.5	D55 x D53	273.5 c
D53	177.6 m			
D53	177.6 m	112.1	D53 x D50	315.0 c
D50	46.6 l			
F08	44.6 l	157.3	F08 x M23	158.7 b
M23	270.1 n			
F23	295.4 n	357.9	F23 x F03	190.3 b
F03	420.4 o			

[1.] Means in each column followed by the same letter are not significantly different at the 5% level
[2.] Theoretical mean of both inbred parents

The lack of information on the mode of resistance inheritance precludes any comment, except that the two best inbreds D57 and F08 produced the best single cross hybrid. Why there is such a low biomass in F23 x F03 (when the parents have such high values), and why D53 x D50 has such a high biomass (when the parents have such low values) is also difficult to explain.

In conclusion, it was demonstrated that at least two distinct and separate resistance mechanisms control the development of stalk

borer larvae feeding in leaf tissue of maize plants. One mechanism resulted in fewer larvae surviving in the plants. The other retarded mass gain of larvae during the active feeding period. These mechanisms were heritable and were observed in single cross hybrids containing a range of inbreds. Some genotypes contained one or both mechanisms, while others were totally susceptible. However, the value of parental data in predicting hybrid levels of resistance was low. This is probably due to the unknown factor of the nutritional status of the plants.

4.1.1.4 Commencement and duration of resistance mechanisms

Previous experiments showed that resistance resulted in fewer larvae surviving in the plants. It was not known when this mechanism was operative, so this experiment was designed to determine the commencement and duration of the mechanism.

(i) Materials and methods

The two single cross hybrids from 4.1.1.1. were chosen for investigation of the resistance. One hybrid (D57 x M06) contained the resistance mechanism which resulted in a lower number of larvae surviving in the leaf tissue. The other hybrid (56 x 58) did not contain any such mechanism.

Experimental plants were randomized in a complete block design, with split plots and 3 replications. The whole plot treatments (5 rows of 10 plants) were the two hybrids and the subplot treatments were five feeding periods. The experiment was planted on 2nd December 1982 and infested with a mean of 20.6 larvae/plant on 29th December 1982.

Sampling of larvae was carried out at five 4-day intervals (4, 8, 12, 16 and 20 days from infestation). Ten plants per treatment in each replication were removed from the field, and the larvae removed, counted and weighed.

(ii) Results and Discussion

(a) Numbers of larvae

Table 4.1.42 summarizes the results of the Analysis of Variance, comparing the mean numbers of larvae removed from both hybrids.

Table 4.1.42. Significance of mean numbers of larvae recovered from the leaf tissue of 2 hybrids at five 4-day intervals

SOURCE OF VARIATION	F	F distribution values	
		5%	1%
Hybrids	267.47**	18.51	98.50
Feeding periods	676.73**	3.01	4.77
Hybrids x feeding periods	105.03**	3.01	4.77

C.V. % Whole Plots = 3.9%
Sub-plots = 6.1%

There were highly significant differences apparent between the numbers of larvae removed from each hybrid, and the numbers of larvae removed at each sampling date. The interaction was also highly significant.

The effect of hybrids on larval numbers

D57 x M06 again had a highly significantly lower number of larvae than was found in 56 x 58. The mean number of larvae/plant over the entire trial was 3.70 for D57 x M06, and 6.39 for 56 x 58 (L.S.D. = 0.68; $P<0.05$). These data again confirmed the presence of a resistance mechanism which reduced larval numbers in D57 x M06.

The effect of feeding period on larval numbers

There was a rapid drop over time in larval numbers. From the initial mean application of 20.6 larvae placed in the funnel of each plant, numbers reduced to an average of 2.34 larvae/plant for both hybrids after 20 days' feeding.

Table 4.1.43. Mean numbers of larvae/plant recovered from the leaf tissue of 2 hybrids at 4-day intervals

DAYS AFTER INFESTATION				
4	8	12	16	20
10.59 a[1.]	5.63 b	3.92 c	2.94 d	2.34 e

[1.] Means followed by the same letter are not significantly different at the 5% level

L.S.D. (5%) = 0.29

Fifty percent of the larvae were already missing only 4 days after infestation. Another 50% of those surviving had also disappeared 4 days later by the 8 day recording. The rate of larval disappearance slowed down by the 12th day, but there was still a significant reduction between the 16-day and 20-day samples.

The effect of the hybrid x feeding period interaction on larval numbers

This highly significant interaction is shown in Table 4.1.44 and Fig. 8.

Table 4.1.44. Mean numbers of larvae recovered at 4-day intervals from the leaf tissue of 2 hybrids

	DAYS AFTER INFESTATION				
	4	8	12	16	20
D57 x M06	7.00 a[1.]	4.66 b	2.90 c	2.65 c	1.67 d
56 x 58	14.19 l	6.59 m	4.94 n	3.22 o	3.02 o
	*	*	*	*	*

L.S.D. 5%	Main effect	Interaction
Hybrids	0.68	0.54
Feeding periods	0.29	0.59

1. Means in each row followed by the same letter are not significantly different at the 5% level

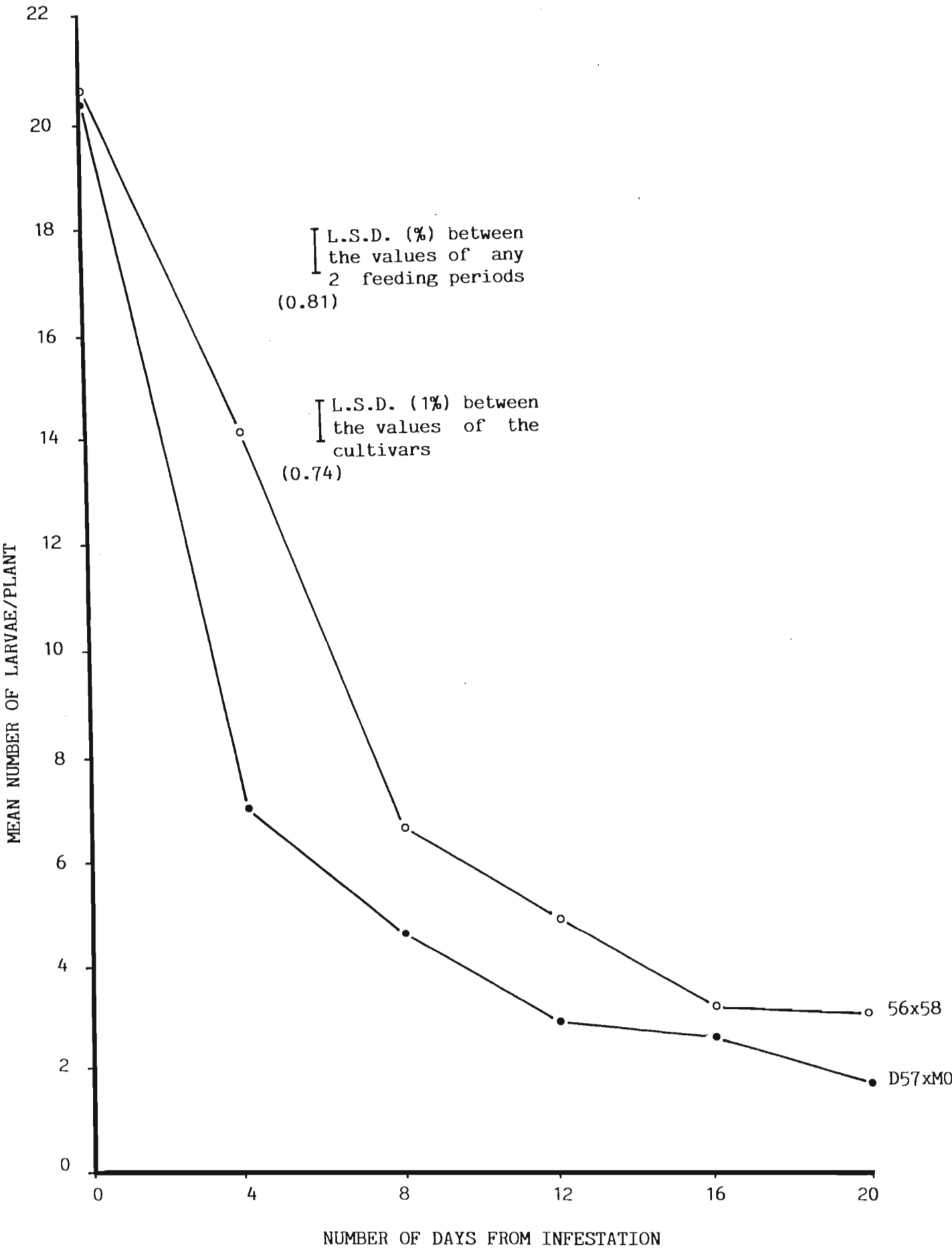


Fig.8. Mean numbers of larvae/plant feeding in the whorl tissue of two hybrids, extracted at 4 day intervals from the date of infestation

Both hybrids showed a significant reduction in larval numbers over time. D57 x M06 showed a far quicker loss of larvae in the first 4 days feeding, losing 66% of all larvae applied to the funnel. This compared with only a 32% loss showed by 56 x 58. This indicated that a resistance mechanism was effective within the first few days feeding by larvae.

From the 4 to 8 day sampling, there was a more rapid reduction in larval numbers in 56 x 58 than in D57 x M06. In 4.1.1.1. (Table 4.1.3) it was concluded that 56 x 58 had no resistance mechanism which resulted in a reduction in larval numbers over the entire larval feeding period of 28 days. However data in Table 4.1.44 and Fig. 8 would indicate a slower acting resistance mechanism present in 56 x 58 which only showed an effect after the 4-day sampling. Competition between larvae can be ruled out as a cause for the reduction, as it was seen in 4.1.1.1 that only more than 17 larvae per plant resulted in a reduction in numbers of larvae. Despite the more rapid reduction in numbers of larvae in 56 x 58 after 8 days, significantly less larvae were present in D57 x M06 over the entire sampling period.

It appears that the resistance mechanism is effective for up to 12 days. It was not known if the resistance mechanism was antibiosis or repellence. This is investigated in 4.1.1.5.

(b) Larval mass

Table 4.1.45 summarizes the results of the Analysis of Variance, comparing the mean larval mass of larvae removed from both hybrids.

Table 4.1.45. Significance of the mean larval mass of larvae recovered from the leaf tissue of 2 hybrids at five 4-day intervals

Source of variation	F	F distribution values	
		5%	1%
Hybrids	391.37**	18.51	98.50
Feeding periods	897.78**	3.01	4.77
Hybrids x feeding periods	59.31**	3.01	4.77

C.V. % Whole Plots = 2.7%
Sub-plots = 7.8%

There were highly significant differences apparent between the mean larval mass of larvae removed from each hybrid, and the mean larval mass of larvae removed at each sampling date. The interaction was also highly significant.

The effect of hybrids on larval mass

Larvae collected from D57 x M06 weighed significantly less than larvae collected from 56 x 58. The mean larval mass over all sampling dates was 10.55 mg and 16.55 mg for D57 x M06 and 56 x 58 respectively (L.S.D. = 1.97, $P<0.05$). These data confirm previous conclusions that, in addition to a resistance mechanism in D57 x M06 which reduced larval numbers, a second resistance mechanism was present which retarded larval development.

The effect of feeding period on larval mass

There was a highly significant, rapid increase in larval mass over time.

Table 4.1.46. Mean larval mass (mg) of larvae recovered from the leaf tissue of 2 hybrids at 4-day intervals

DAYS AFTER INFESTATION				
4	8	12	16	20
1.05	5.02	10.35	17.41	33.94
a[1.]	b	c	d	e

[1.] **Means followed by the same letter are not significantly different at the 5% level**

L.S.D. (5%) = 1.29

The largest gain was in the last 4 days, due to increased larval size and concomitant ability to consume more food.

The effect of hybrid x feeding period interaction on larval mass

This highly significant interaction is shown in Table 4.1.47 and Figure 9.

Table 4.1.47. Mean larval mass (mg) of larvae recovered from the leaf tissue of 2 hybrids at 4 day intervals

	DAYS AFTER INFESTATION				
	4	8	12	16	20
D57 x M06	0.94 a[1.]	4.44 b	8.26 c	13.52 d	25.61 e
56 x 58	1.15 m	5.60 n	12.44 o	21.31 p	42.27 q
	N.S.	N.S.	*	*	*

[1.] Means in each row followed by the same letter are not significantly different at the 5% level

L.S.D. 5%	Main effect	Interaction
Hybrids	1.97	1.83
Feeding periods	1.29	1.76

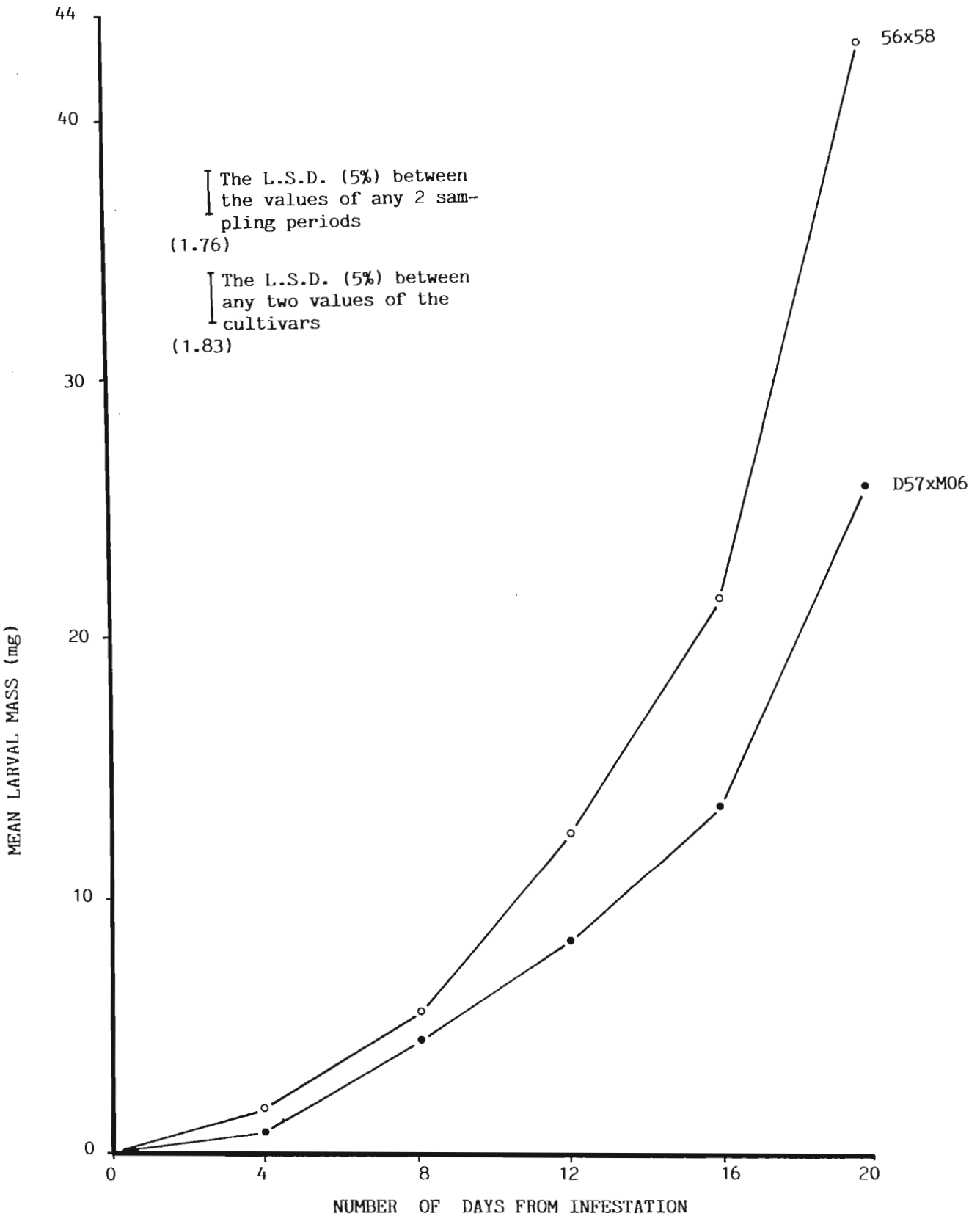


Fig.9. Mean larval mass of larvae feeding in the whorl tissue of 2 hybrids, extracted at 4 day intervals from infestation

Larvae feeding in both hybrids showed a similar mass gain by the 4 and 8 day samples, with no significant differences between the hybrid values. At the 12 day sample, however, a highly significant difference was apparent. The difference between the mean larval mass found in each hybrid increased substantially up to the 20 day sample. At this sampling date, larvae feeding in 56 x 58 weighed 65% more than larvae feeding in D57 x M06. The resistance mechanism affecting mass gain was obviously still operative at the 20 day sampling. The percentage increase in mass shown by larvae from sampling date to sampling date is shown in Table 4.1.48.

Table 4.1.48. Percentage increase in mean larval mass from one sampling date to the next for larvae feeding in the leaf tissue of 2 hybrids

	SAMPLING PERIOD			
	4-8 days	8-12 days	12-16 days	16-20 days
D57 x M06	372.3	93.6	63.6	89.4
56 x 58	386.9	122.1	71.3	98.3

Larvae feeding in D57 x M06 increased in mass at a slower rate than larvae feeding in 56 x 58.

(c) Larval biomass

Table 4.1.49 summarizes the results of the Analysis of Variance, comparing the mean larval biomass/plant of larvae removed from both hybrids.

Table 4.1.49. Significance of mean larval biomass/plant of larvae recovered from the leaf tissue of 2 hybrids at five 4-day intervals

Source of variation	F	F distribution values	
		5%	1%
Hybrids	9886.31**	18.51	98.50
Feeding periods	4599.85**	3.01	4.77
Hybrids x Feeding periods	1323.33**	3.01	4.77

C.V.% Whole Plots = 7.4%
Sub-plots = 9.6%

As expected from the highly significant values for larval numbers and mass, there were highly significant differences apparent between the mean larval biomass/plant found in the two hybrids. The increases in biomass over the five sampling dates were also highly significant, as was the interaction.

The effect of hybrids on larval biomass

The mean larval biomass/plant recovered from each hybrid over five sampling periods was 25.96mg and 62.27mg for D57 x M06 and 56 x 58 respectively. The difference between these values was highly significant (L.S.D. = 11.44, $P<0.05$).

The effect of feeding period on larval biomass

Highly significant increases in larval biomass occurred with time.

Table 4.1.50. Mean larval biomass/plant (mg) recovered from the leaf tissue of 2 hybrids at five 4-day intervals

DAYS AFTER INFESTATION				
4	8	12	16	20
11.48 a[1.]	28.74 b	42.73 c	52.33 d	85.30 e

[1.] **Means followed by the same letter are not significantly different at the 5% level**

L.S.D. (5%) = 5.15

The effect of hybrid x feeding period on larval biomass

This highly significant interaction is shown in Table 4.1.51 and Fig. 10.

Table 4.1.51. Mean larval biomass/plant (mg) of larvae recovered from the leaf tissue of 2 hybrids at 4-day intervals

	DAYS AFTER INFESTATION				
	4	8	12	16	20
D57 x M06	6.60 a[1.]	20.75 b	23.99 b	35.83 c	42.65 c
56 x 58	16.37 m	36.73 n	61.48 o	68.83 o	127.94 p
	*	*	*	*	*

L.S.D. 5%	Main effect	Interaction
Hybrids	11.44	7.29
Feeding periods	5.15	8.63

[1.] **Means in each row followed by the same letter are not significantly different at the 5% level**

The significant difference between larval biomass/plant feeding in the hybrids after 4 days was due to the highly significant reduction in larval numbers found in D57 x M06 (Table 4.1.44). After 4 days, there was a non significant difference between larval mass in each hybrid, so mass did not contribute substantially to the biomass differences.

The highly significant difference between larval biomass found in both hybrids thereafter is due to a combination of fewer and

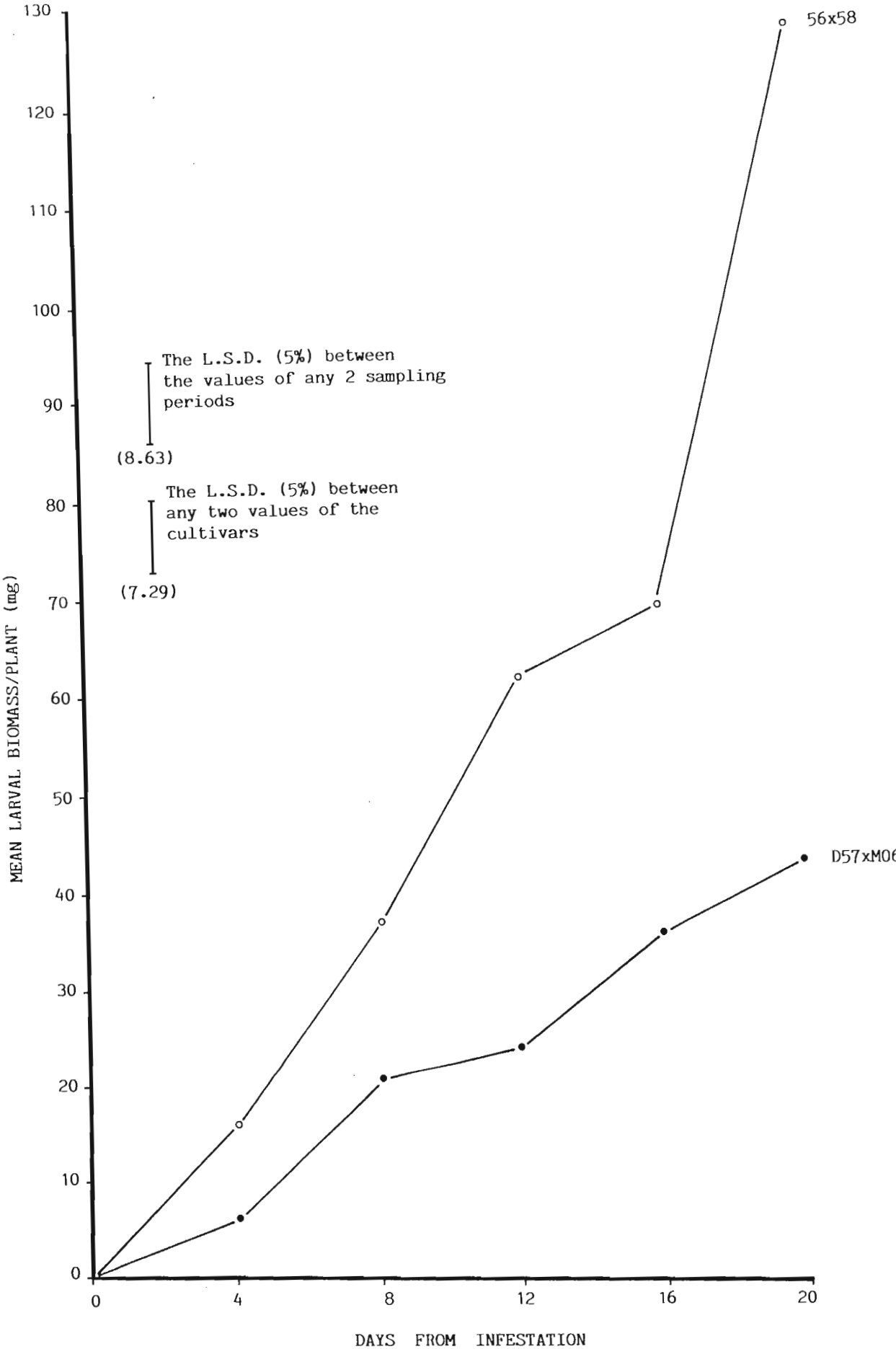


Fig. 10. Mean larval biomass/plant of larvae feeding in the whorl

lighter larvae in D57 x M06 than found in 56 x 58. The difference in larval numbers between hybrids at the 8 day sampling was fairly small, but significant. Thereafter, larvae feeding in 56 x 58 grew at a much faster rate than the larvae feeding in D57 x M06. This resulted in a much larger final larval biomass feeding in 56 x 58 than in D57 x M06.

In D57 x M06 the increase in larval biomass over time was slight. There were no significant differences in biomass between the values recorded at the 8 and 12 day samplings. There were also no significant differences between the 16 and 20 day samplings. Larval biomass increased significantly in 56 x 58 up to the 12 day sampling. There was no significant difference between the values of the 12 and 16 day samplings, but there were between the 16 and 20 day samplings.

4.1.1.5 Leaf resistance - mechanisms

The objective of this experiment was to determine whether low numbers of larvae surviving in resistant maize germplasm resulted from antibiosis or repellence. Antibiosis results in the death of larvae in the plant, whereas repellence results in emigration from the plants. In both instances, a reduction in larval numbers occurs.

(i) Materials and methods

Six previously-screened inbred lines were selected because of their different effects on numbers of maize stalk borer larvae feeding in whorl tissue. The inbreds F03, D53 and M23 had high larval numbers feeding in the whorl tissue and were classified as susceptible. Inbreds F08, D57 and D55 had low larval numbers feeding in the whorl tissue and were classified as resistant. It was not known whether the low numbers were due to antibiosis or repellence.

The Experiment was planted on 18th November 1983, as a randomized complete block design with four replications. Each plant of the experimental inbreds was surrounded by susceptible control plants

of the susceptible inbred 56. This inbred had always contained high numbers of larvae in previous experiments. One border row of 56 surrounded the experiment (see diagram). The next row had alternately a border plant of 56 then a plant of the inbred under investigation, then two border plants of 56, then the inbred followed again by two 56 border plants. This pattern was repeated along the row for 30 plants, ensuring that each experimental inbred was totally surrounded by susceptible plants of 56. A total of 50 plants of each inbred was infested per replication, with 400 border plants of 56 that were not infested.

```
X  X  X  X  X  X .......30 Plants (total)
X  0  X  X  0  X
X  X  X  X  X  X
X  X  X  X  X  X
X  0  X  X  0  X
X  X  X  X  X  X
.
.
.

15  rows  total
X = inbred 56
0 = experimental inbred
```

The experimental inbreds were infested on 22nd December with a mean of 18.6 larvae per plant. If the resistance mechanism was one of repellence, larvae would move out of the infested inbreds and into the susceptible plants of 56. These plants would then show leaf damage several days later. If no damage appeared in the plants of 56, then migration was assumed to have not occurred.

To determine when, and for how long, migration occurred, assessment of leaf damage was carried out in the uninfested border plants 7, 14 and 21 days after infestation. Visible damage normally shows up about 5 days after larvae have commenced feeding in the whorl.

After 17 days feeding, all the infested experimental plants were dissected and the larvae therein counted and weighed. Larvae that caused the damage in the border plants at the 21 day sampling would have already moved out of the infested inbreds after 16 days feeding. Destructively sampling the infested inbreds after 17 days feeding would therefore not have affected the immigration-caused leaf damage in the border plants recorded after 21 days feeding.

(ii) Results and discussion

(a) Larval emigration

Table 4.1.52 summarizes the results of the Analysis of Variance, comparing the mean percentage infestation recorded in the border plants of inbred 56 surrounding the infested inbreds. There were highly significant differences apparent between the percentage infestations in the plants surrounding the different inbreds. There were no significant differences evident between feeding periods, nor was the interaction significant.

Table 4.1.52. Significance of mean percentage infestation of border plants of 56 surrounding the infested inbreds

SOURCE OF VARIATION	F		F distribution values	
			5%	1%
Inbreds	118.24	**	2.90	4.56
Feeding period	0.55	N.S.	3.27	5.25
Inbreds x feeding period	0.43	N.S.	2.20	2.90

C.V.% Whole Plots = 7.4%
Sub-plots = 3.6%

The effect of inbreds on larval migration

There were significant differences between inbreds with regard to infestation of the surrounding plants of 56. These differences are shown in Table 4.1.53 (mean of three counts, taken after 7, 14 and 21 days feeding).

Table 4.1.53. Emigration of larvae out of six inbreds, expressed as the percentage infestation of border plants surrounding the infested inbreds[1.]

INBRED					
D57	D53	M23	F03	F08	D55
8.3a[2.]	10.5a	12.4a	12.5a	66.5b	71.0b

[1.] Mean of three counts, taken after 7, 14 and 21 days feeding
[2.] Means followed by the same letter are not significantly different at the 5% level

L.S.D. (5%) = 8.3

These 6 inbreds had been chosen specifically for this experiment because of their effects on larval numbers. Three of them (D57, F08, D55) had previously shown a resistance mechanism which reduced larval numbers. The other three (D53, M23, F03) had shown no resistance which reduced larval numbers.

There was, as expected, very little migration of larvae out of the susceptible group (D53, M23, F03) into the surrounding plants of 56. The data from the resistant group (D57, F08, D55), however, did not completely conform to expectations. Only two inbreds, F08 and D55, showed larval migration (repellence) into the border plants (66.5% and 71.0% respectively). The border plants surrounding D57 were only 8.3% infested, indicating that larvae were not repelled by feeding in D57. Only F08 and D55 had a repellence or non-preference resistance mechanism. The resistance mechanism shown by D57 was only elucidated when larvae were dissected out of the plants after 17 days feeding

(see below).

The effect of feeding period on larval migration

Infestation of the border plants was recorded as a cumulative percentage infestation, taken 7, 14 and 21 days ater infestation. As 96.5% of that emigration occurred within the first 7 days of feeding, there were no significant differences between the figures recorded at each sampling date.

Table 4.1.54. Larval emigration from six inbreds into surrounding border plants expressed as the cumulative mean percentage infestation, taken after 7, 14 and 21 days feeding

DAYS AFTER INFESTATION		
7	14	21
29.5a[1.]	30.6a	30.6a

[1.] Means followed by the same letter are not significantly different at the 5% level

L.S.D. (5%) = 1.2

The effect of the inbreds x time interaction on larval migration

Table 4.1.55. Larval emigration from six inbreds into surrounding border plants expressed as the cumulative mean percentage infestation, taken after 7, 14 and 21 days feeding

DAYS AFTER INFESTATION	INBREDS					
	D57	D53	M23	F03	F08	D55
7	7.7a[1]	9.9a	11.8a	12.2a	65.0b	70.4b
14	8.6l	10.9l	12.7l	12.7l	67.2m	71.3m
21	8.6p	10.9p	12.7p	12.7p	67.2q	71.3q
	N.S.	N.S.	N.S.	N.S.	*	N.S.

[1] Means in rows followed by the same letter are not significantly different at the 5% level

L.S.D. 5%	Main effect	Interaction
Inbreds	8.3	1.6
Days	1.2	7.9

Most of the migration occurred within the first 7 days feeding. No significant interaction occurred between inbreds and time, except for F08, where slightly more emigration occurred after the 7 day sampling. In all inbreds, there was no increase in migration after the 14 day sampling. The resistance mechanism is obviously a short lived, early acting mechanism.

(b) Numbers of larvae

Table 4.1.56 summarizes the result of the Analysis of Variance, comparing the mean numbers of larvae recovered from each inbred after 17 days feeding.

Table 4.1.56. Significance of mean numbers of larvae/plant, recovered from 6 inbreds, after 17 days feeding

SOURCE OF VARIATION	F	F distribution values	
		5%	1%
Inbreds	72.64**	2.90	4.56

C.V.% Plots = 9.6%

Significant differences occurred between numbers of larvae recovered from each inbred after 17 days feeding.

Table 4.1.57. Mean numbers of larvae/plant recovered from 6 inbreds after 17 days feeding

INBREDS					
D57	F08	D55	F03	M23	D53
2.07 a[1.]	2.42 a	2.65 a	4.82 b	5.05 b	5.58 b

[1.] Means followed by the same letter are not significantly different at the 5% level

L.S.D. (5%) = 0.75

In 4.1.1.2 it was recorded that D57, F08 and D55 had low populations of larvae. These data were confirmed in this experiment. In the case of F08 and D55 this was expected, as larvae had migrated out of these inbreds (Table 4.1.57). The inbred D57 did not have larvae migrating out of the plants, yet showed a low number of larvae surviving. The conclusion is that low larval numbers in D57 were caused by antibiosis and not repellence.

There are thus two different resistance mechanisms (repellence and antibiosis) which reduce the population of stalk borer feeding in whorl tissue. The repellent mechanism acts within 7 days of commencement of feeding. It was not ascertained whether larvae eclosing on these plants under natural conditions would be repelled before or after feeding. As leaf damage was recorded on F08 and D55, it would indicate that repellence occurred after feeding had commenced.

(c) Larval mass

Table 4.1.58 summarizes the result of the Analysis of Variance, comparing the mean larval mass recovered from each inbred after 17 days feeding.

Table 4.1.58. Significance of mean larval mass of larvae recovered from 6 inbreds after 17 days feeding

SOURCE OF VARIATION	F	F distribution values	
		5%	1%
Inbreds	664.23**	2.90	4.56

C.V.% Plots = 15.8%

The significant differences between mean larval mass of larvae feeding in the 6 inbreds are shown in Table 4.1.59.

Table 4.1.59. Mean larval mass (mg) of larvae recovered from 6 inbreds after 17 days feeding

INBREDS					
D57	D53	F08	D55	M23	F03
11.95 a[1.]	12.37 a	12.85 a	18.37 a	29.70 b	43.95 c

[1.] **Means followed by the same letter are not significantly different at the 5% level**

L.S.D. (5%) = 7.08

In addition to having antibiosis, D57 also had a longer lasting resistance mechanism which retarded the development of larvae feeding in the whorl. These larvae were only 27% of the mass of larvae feeding in F03. D53 showed neither antibiotic nor repellent effect on larvae (Table 4.1.57). It did, however show a retarding effect on growth of larvae. This confirms previous findings that the resistance affecting larval growth is distinct and separate from the two mechanisms reducing numbers of early instar larvae feeding in whorl tissue.

F08 and D55 both showed a repellent effect on larvae (Table 4.1.57) and show a resistance effect on larval growth. Although both appear to have this mechanism, D55 did not have as strong an effect as F08.

Neither M23 nor F03 had any repellent or antibiotic effect on larvae, nor did they contain any mechanism which retarded larval growth. It appears from these and other data (4.1.1.2) that the resistance which retards larval growth is a quantative effect of several genes, as the mean larval mass ranged from 11.9 mg/larva for D57 to 43.9 mg/larva (F03). In 4.1.1.2 larval sampling in

M23 after 15 days feeding showed that a resistance mechanism was operative in reducing larval mass. However, by the 25 day sample, this resistance was negligible, and mass gain of larvae feeding in M23 during this period was one of the highest of all inbreds. It is possible therefore that M23 only has resistance for a short while, and in this present experiment M23 could already have been losing that resistance by the 17 day sampling date. It is probable that, in addition to polygenic inheritance of the resistance mechanism (i.e. presence or absence), the initiation and termination of the mechanism could also be controlled polygenically.

(d) Larval biomass

Table 4.1.60 summarizes the result of the Analysis of Variance, comparing the mean larval biomass/plant recovered from each inbred after 17 days feeding.

Table 4.1.60. Significance of mean larval biomass/plant recovered from 6 inbreds, after 17 days feeding

SOURCE OF VARIATION	F	F distribution values	
		5%	1%
Inbreds	60.03**	2.90	4.56

C.V. % Plots = 21.7%

The significant differences between mean larval biomass/plant recovered from each inbred are due to various combinations of the 3 resistance mechanisms occurring in the different inbreds.

Table 4.1.61. Summary of the mean larval biomass/plant in each inbred and the occurrence of the 3 resistance mechanisms effecting larval survival

INBRED	MEAN LARVAL BIOMASS (mg) PER PLANT	RESISTANCE MECHANISM		
		Repellence	Antibiosis	Mass gain
D57	24.7a[1]	No	Yes	Yes
F08	31.8ab	Yes	No	Yes
D55	48.8ab	Yes	No	Yes
D53	68.6b	No	No	Yes
M23	150.2c	No	No	(Yes)[2]
F03	212.0d	No	No	No

L.S.D. (5%) = 40.4

[1] Means followed by the same letter are not significantly different at the 5% level

[2] See Page 131 for explanation

As the mean larval biomass/plant is in direct proportion to the amount of damage caused to the plant, possession of any of these resistance mechanisms would result in less damage being caused.

(iii) Seasonal Comparisons

These 6 inbreds were also investigated in 4.1.1.2. The comparison of data from both experiments is shown in Table 4.1.62.

Table 4.1.62. Comparison over seasons of data on larval numbers, mass and biomass of larvae recovered from 6 inbreds

INBRED	MEAN NO. OF LARVAE/PLANT		MEAN MASS (mg) PER LARVA		MEAN BIOMASS /PLANT (mg)	
	1983[1.]	1982[2.]	1983	1982	1983	1982
D57	2.1a[3.]	2.3d	11.9g	2.7j	24.7m	6.2s
F08	2.4a	1.8c	12.8g	2.5j	31.8mn	4.6s
D55	2.6a	2.0d	18.4g	2.9j	48.8mn	5.8s
D53	5.6b	4.1ef	12.4g	5.8j	68.6n	23.4s
M23	5.0b	4.6f	29.7h	6.0j	150.2o	25.5s
F03	4.8b	3.4e	43.9i	22.2k	212.0p	71.9t

1. After 17 days feeding (4.1.1.5 - 1983)
2. After 15 days feeding (4.1.1.2 - 1982)
3. Means in each column followed by the same letter are not significantly different at the 5% level

Mean numbers of larvae/plant were similar over seasons. There are two distinguishable groups: the resistant group of D57, F08 and D55, and the susceptible group of D53, M23 and F03.

Larvae were heavier in 1983, but had fed for two days longer than larvae in 1982. The extra two days feeding would have enabled them to have gained the increased mass recorded in 1983.

Correlation between seasons was good for all inbreds, except for M23, which showed a heavier mass gain in 1983 than in 1982. The inbred F03 had the heaviest larvae in both experiments.

Mean larval biomass/plant, although showing higher values in 1983 than in 1982 due to heavier larval mass, also showed good correlation over seasons. M23 however differed substantially over seasons due to a much heavier mean larval mass in 1983.

With the genotypes studied so far, a maize genotype has not yet been found to have all 3 types of resistance (repellence, antibiosis and growth retardation). Some genotypes have good resistance involving repellence and growth retardation. Others have resistance acting by way of antibiosis and growth retardation. Both groups appear equally effective in reducing insect biomass. One inbred contained only the resistant factor affecting mass gain, and lacked the resistant factors affecting larval numbers. The control inbred F03 is only one of hundreds of inbreds screened and found to be highly susceptible.

4.2 STEM RESISTANCE: DEVELOPMENT OF LIFE STAGES

4.2.1 Development of larvae in different maize genotypes

In addition to feeding in leaf tissue, larvae can spend a considerable amount of time feeding in stem tissue (Plate 12). Larvae that have attained the pre-pupal stage while feeding in leaf tissue will migrate out of the whorl and hollow out a cavity in the stem. This cavity has a volume of only several ml's, and very little feeding occurs; its prime purpose is to accommodate the pupa. However, extensive stem feeding will occur in maize that is infested at a late growth stage. After only a few days feeding in the whorl, larvae will be forced out by the emerging tassel. They will still have several weeks feeding to complete, and this takes place in the stem.

Stem feeding obviously causes a reduction in sap flow. The more severe the damage, the greater the interuption to the essential plant processes (Plates 14,15). If resistance in stem tissue could be incorporated into maize hybrids, obvious benefits would accrue. The following experiment was carried out to determine whether there were differences in stem tissue resistance, and how the resistance affected *B. fusca*. In Chapter 5, the effect on the maize plants of larvae feeding in stem tissue of several maize genotypes is discussed.

(i) Materials and methods

Three single cross hybrids were chosen to give a range of larval responses to the maize genotypes. Their selection was based on larval responses in stems of several hybrids assessed in experiments carried out in 1982/83. The hybrid F07 x F09 was chosen as the most resistant of the three maize genotypes, F09 x F08 was intermediate, and D50 x K80 was included as the most susceptible hybrid.

Experimental plants were randomized in a complete block design, with split plots and three replications. The whole plot treatments (one row of 10 plants spaced 22,5cm apart) were eight

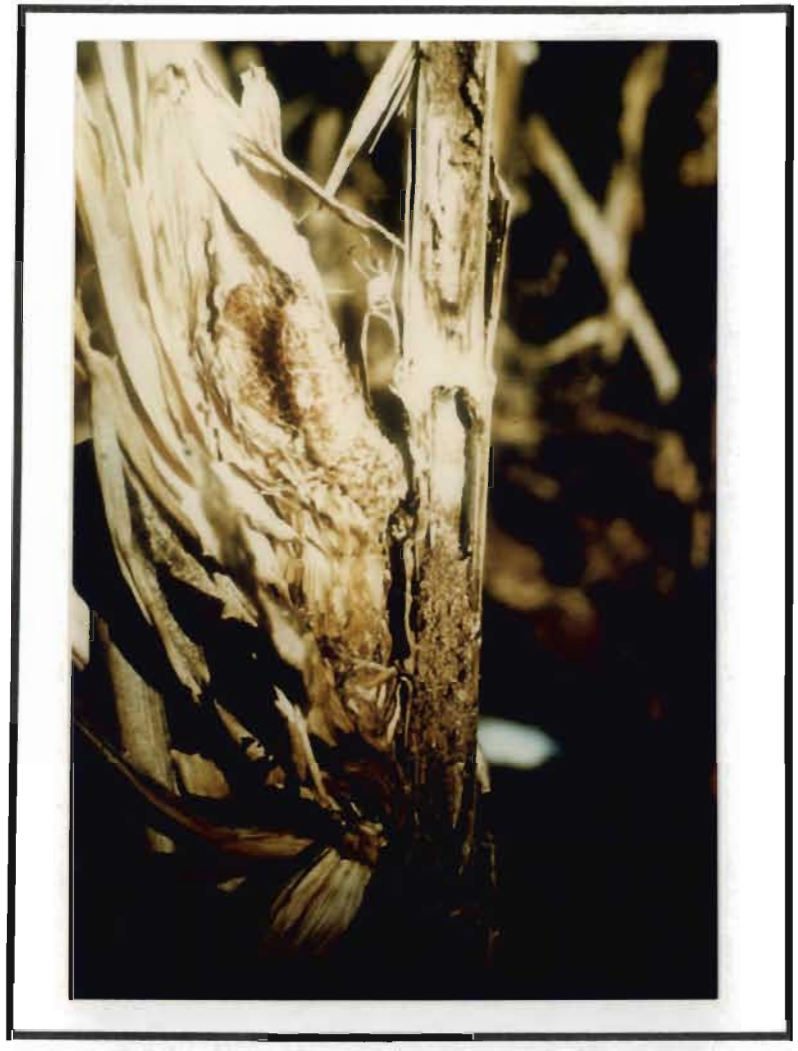


Plate 14. Loss of yield due to extensive stem damage below the ear.

Plate 15. "Dead heart" caused by early stem boring in young plant.

larval feeding periods (10, 17, 24, 31, 38, 45, 52 and 59 days feeding). The 3 hybrids constituted the sub-plots. The infested rows were separated from each other by two uninfested double planted barrier rows. This arrangement prevented larval migration between infested rows.

The seeds were planted on 1st November 1983, and all plants were infested with a mean of 19,7 larvae/plant for the whole trial, applied with a "bazooka" on 12th December 1983.

Sampling of larvae and pupae was carried out by dissecting the 10 plants in each of eight treatment rows at weekly intervals after 10, 17, 24, 31, 38, 45, 52 and 59 days feeding. Data were recorded on numbers, mass and biomass of each life-stage present, site of feeding (leaf tissue or stem), and time of occurrence of stem boring and pupation.

(ii) Results and discussion

(a) Larval and pupal data: numbers of insects

There was a highly significant ($P<0.01$) difference between the numbers of larvae and pupae recovered from each hybrid. There was also a highly significant ($P<0.01$) difference in the numbers of larvae and pupae recovered at each sampling date. The interaction between the two sources of variation was also highly significant ($P<0.01$).

Table 4.2.1 summarizes the results of the Analysis of Variance comparing the mean numbers of larvae and pupae removed from each hybrid, at weekly intervals.

Table 4.2.1. Significance of mean numbers of larvae and pupae recovered from 3 single cross hybrids at weekly intervals

SOURCE OF VARIATION	F	F distribution values	
		1%	5%
Feeding periods	65.04**	4.32	2.77
Hybrids	82.75**	5.39	3.32
Feeding periods x hybrids	2.99**	2.84	2.09

C.V. % Whole Plots = 6.8%
Sub-plots = 10.8%

The effect of feeding period on insect numbers

There were highly significant ($P<0.01$) differences between sampling dates, with regard to the numbers of larvae and pupae removed from the hybrids at each sampling interval.

Table 4.2.2. Mean numbers of larvae and pupae/plant recovered at weekly intervals, averaged over 3 hybrids

Feeding Period (Days)							
10	17	24	31	38	45	52	59
12.8a[1]	11.9a	10.8b	8.8c	7.7d	6.2e	6.0e	5.9e

[1] Means followed by the same letter are not significantly different at the 5% level

L.S.D. (5%) = 1.0

As observed in previous experiments, the largest reduction in numbers of larvae occurred in the first 10 days of feeding. This reduction was due to normal high mortality of first instar larvae caused by various factors (predation, migration, adverse weather). In addition, reduction in larval numbers was due also to resistance in F07 x F09 which caused either larval death or migration (see Table 4.2.3). There was no significant drop in numbers between the 10-and 17-day samplings. When larvae reach the pre-pupal stage, they migrate out of the whorl into the stem just prior to pupation. Stem boring was observed at the 24 and 31 day sampling (see (d) stem boring). This migration presumably resulted in more significant larval losses. Numbers of larvae stabilised from the 45 to 59 day sampling as migration had ceased, and all larvae had either pupated or were still feeding in the stems.

The effect of hybrids on insect numbers

There were highly significant differences ($P<0.01$) between the numbers of larvae and pupae recovered from the 3 hybrids, averaged over the 8 sampling dates (Table 4.2.3)

Table 4.2.3. Mean numbers of larvae and pupae/plant recovered from 3 single cross hybrids over 8 sampling dates

HYBRID	INSECT NUMBERS
F07 x F09	7.0 a[1.]
F09 x F08	8.8 b
D50 x K80	10.5 c

1. Means followed by the same letter are not significantly different at the 5% level

L.S.D. (5%) = 0.8

Discussion of these data is included below in the interaction discussion.

The effect of feeding period x hybrid interaction on numbers of larvae and pupae/plant

Table 4.2.4 shows the highly significant ($P<0.01$) interaction between these two variates.

Table 4.2.4. **Mean numbers of larvae and pupae/plant recovered from hybrids at weekly intervals**

	FEEDING PERIOD (DAYS)							
Hybrid	10	17	24	31	38	45	52	59
F07 x F09	10.6a[1]	9.7e	8.8h	7.5k	4.8n	5.0r	4.9u	4.8x
F09 x F08	13.2b	12.9f	11.5i	7.6k	7.1o	6.4rs	5.8u	6.4y
D50 x K80	14.8c	13.3f	12.2i	11.3l	11.3p	7.4s	7.3v	6.7y

[1] Means in columns followed by the same letter are not significantly different at the 5% level

L.S.D. (5%)	Main effect	Interaction
Feeding periods	1.0	1.6
Hybrids	0.7	1.7

There were significant differences in numbers of larvae and pupae recovered from each hybrid. F07 x F09 always had the fewest insects/plant and D50 x K80 always had the most. This was due to the resistance in F07 x F09 which caused an initial reduction in numbers of larvae in the first few days of feeding. After the 10 day sampling, larval losses showed a similar trend for all hybrids.

There were some small but significant reductions in larval numbers as the feeding period increased. Larvae developed quickly in F09 x F08 and D50 x K80 and began migrating out of the plant whorls after only 24 days feeding (see Fig.11). A resistance factor present in F07 x F09 delayed development of larvae feeding in this hybrid (see Table 4.2.8.), resulting in migration and stem boring only after 31 days feeding. This migration ceased after 38 days feeding when all larvae that were recovered from the plants were found in the stem tissue. During the period 24 to 38 days feeding, the largest loss in numbers of larvae occurred in F07 x F09 (47,7% loss), compared with a loss of 38,3% in F09 x F08 and a loss of only 7,37% in D50 x K80. This may indicate that the stem tissue of D50 x K80 was a more acceptable food source than that of the other two hybrids. Larvae boring into the stems of F07 x F09 and F09 x F08 may have been repelled, resulting in a higher larval mortality. However, this aspect was not investigated. A large unexplained loss of larvae occurred in D50 x K80 during the period 38 to 45 days. Thereafter, since all larvae had stopped migrating out of the whorl and were all found in the stem, numbers of larvae and pupae remained fairly constant.

(b) Larval and pupal data: insect mass

Table 4.2.5 summarizes the results of the Analysis of Variance, comparing the mean larval and pupal mass, recovered from 3 hybrids, over eight weekly sampling intervals.

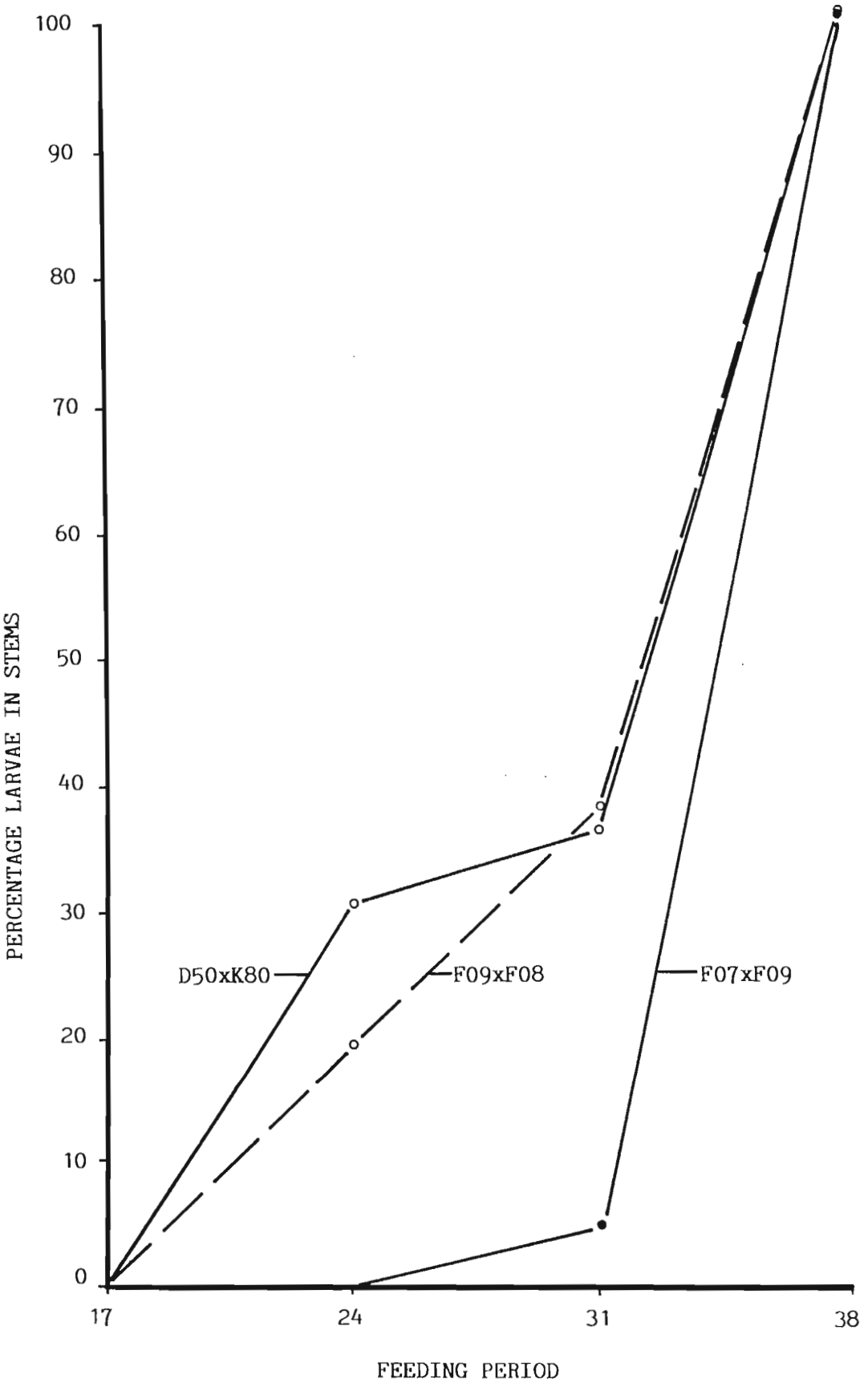


Fig. 11. Percentage larvae found feeding in stem tissue in three single cross maize hybrids

Table 4.2.5. Significance of mean insect mass of larvae and pupae recovered from 3 hybrids, at eight weekly intervals

SOURCE OF VARIATION	F	F distribution values	
		1%	5%
Feeding periods	1537.30**	4.32	2.77
Hybrids	317.85**	5.39	3.32
Feeding periods x Hybrids	77.57**	2.84	2.09

C.V. % Whole Plots = 3.7%
Sub-plots = 4.9%

Larvae gained mass rapidly with time, and there were highly significant ($P<0.01$) differences evident in the insect mass sampled from each hybrid, and at each sampling date. The interaction was also highly significant ($P<0.01$).

The effect of feeding period on insect mass

Table 4.2.6. Mean insect mass (mg) of larvae and pupae/plant recovered at eight weekly intervals, averaged over 3 hybrids

FEEDING PERIOD (DAYS)							
10	17	24	31	38	45	52	59
1.3a[1.]	7.3a	35.2b	122.2c	213.5d	285.6e	304.6f	302.8f

[1.] Means followed by the same letter are not significantly different at the 5% level

L.S.D. (5%) = 10.2

Larvae increased rapidly in mass during the larval feeding period. Pupation was recorded soon after 38 days feeding in F09 x F08 and D50 x K80 (see (e) pupation). Pupation was delayed in F07 x F09 and was recorded only after 45 days. As more larvae pupated after this period (and therefore were at maximal mass), the mass increase during the following 2 weeks was not so marked. This is discussed fully below in the discussion on the interaction.

The effect of hybrids on insect mass

There were highly significant ($P<0.01$) differences evident in the mean mass of larvae and pupae removed from the hybrids (Table 4.2.7). Hybrid F07 x F09 had much smaller insects than the other two hybrids which had an almost identical insect mass.

Table 4.2.7. Mean insect mass (mg) of larvae and pupae recovered from 3 hybrids averaged over 8 sampling dates

HYBRID	MEAN MASS (mg)
F07 x F09	126.7 a[1.]
F09 x F08	174.4 b
D50 x K80	177.3 b

[1.] **Means followed by the same letter are not significantly different at the 5% level**

L.S.D. (5%) = 4.7

These data (the average mass of larvae and pupae recovered from leaf and stem tissue) confuse the real picture, as resistance mechanisms in leaf tissue of F07 x F09 caused large significant differences between larval mass feeding in the hybrids while larvae were feeding in leaf tissue. These differences diminished once larvae commenced feeding in stem tissue, as larvae gained mass rapidly. Also, larvae commenced stem feeding earlier in F09 x F08 and D50 x K80 than in F07 x F09. This significantly influenced the mean insect mass averaged over the entire feeding

period, and is discussed fully under the interaction.

The effect of feeding period x hybrid interaction on insect mass

Table 4.2.8. Mean insect mass (mg) of larvae and pupae recovered from 3 hybrids at eight weekly intervals

	FEEDING PERIOD (DAYS)							
Hybrids	10	17	24	31	38	45	52	59
F07 x F09	0.6a[1]	3.3c	8.1e	22.1h	105.5l	258.0o	301.9r	299.5t
F09 x F08	1.5a	9.1c	46.1f	173.7i	264.6m	299.3p	306.1r	295.0t
D50 x K80	1.8a	9.6c	51.4f	170.7i	270.6m	299.5p	305.7r	308.9t

[1] Means in columns followed by the same letter are not significantly different at the 5% level

L.S.D. (5%)	Main effect	Interaction
Feeding periods	10.2	13.3
Hybrids	4.7	14.7

Larvae feeding in F07 x F09 were significantly smaller than larvae feeding in the other two hybrids at the 24, 31, 38 and 45 day samplings. Up to the 38 day sampling larvae fed in leaf tissue. When larvae commenced feeding in stem tissue of F07 x F09 (after the 31 day sampling), they gained mass at a rapid rate, finally equalling the mass of larvae feeding in the other two hybrids at the 52 day sampling date (See Fig.12). This was a direct consequence of there being no apparent resistance mechanism present in the stem tissue of F07 x F09, and the larvae therefore fed unhindered to maturity.

In Fig.12, the increase in mass of larvae feeding in F07 x F09 is expressed as a percentage of the mass of larvae and pupae found in D50 x K80. During the first 24 days feeding, 100% of

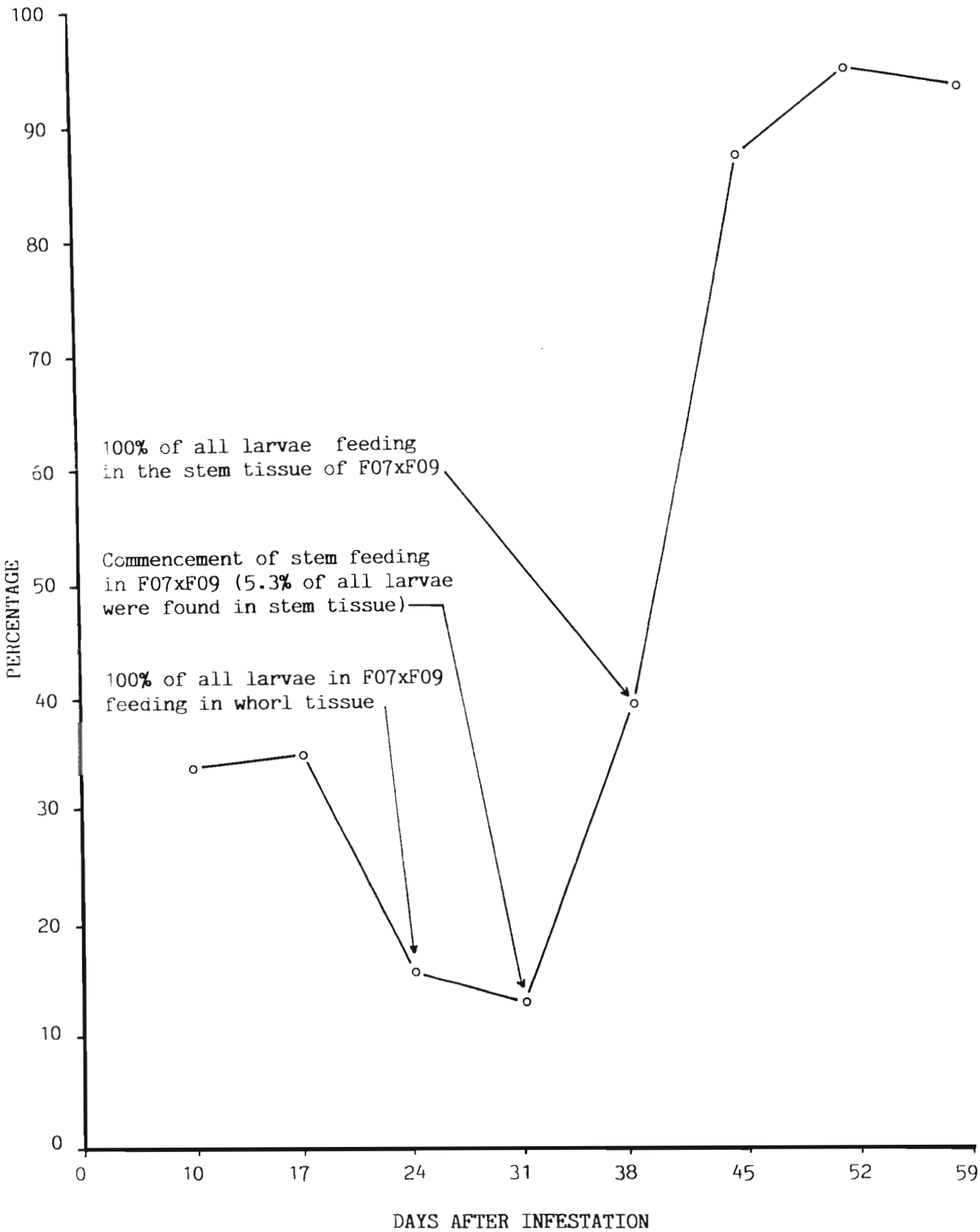


Fig. 12. Mean insect mass of larvae and pupae in F07 x F09, expressed as a percentage of the mass of larvae and pupae in D50 x K80

all larvae in F07 x F09 fed on whorl tissue which contained the resistant factor affecting mass gain. The effect of this resistance can be seen as a reduction of the relative mass. At the 31 days sampling, 5.3% of larvae feeding in F07 x F09 had commenced feeding in the stem. At this sampling date, these larvae were only 12.9% of the mass of larvae feeding in D50 x K80. Larvae feeding on susceptible stem tissue of F07 x F09 rapidly gained mass. After 38 days feeding, 100% of all larvae in F07 x F09 were feeding in stem tissue, and weighed 38.9% of the mass of larvae feeding in D50 x K80. Thereafter, larvae rapidly gained mass, and after 52 days feeding, larvae were 98.7% of the mass of larvae feeding in D50 x K80. This clearly illustrated that leaf and stem tissue of a single hybrid varied in the levels of resistance or susceptibility to *B. fusca* larvae. As the majority of larvae feeding in commercial maize complete their life cycles in stem tissue, with varying amounts of stem feeding, the assessment of the levels of resistance in stem tissue must form an integral part of any HPR investigation.

(c) Larval and pupal data: insect biomass

Table 4.2.9 summarizes the results of the Analysis of Variance, comparing the mean insect biomass/plant for 3 hybrids, recovered during 8 weekly sampling intervals.

Table 4.2.9. Significance of mean biomass/plant of larvae and pupae recovered at weekly intervals from 3 hybrids

SOURCE OF VARIATION	F	F distribution values	
		1%	5%
Feeding periods	113.43**	4.32	2.77
Hybrids	165.98**	5.39	3.22
Feeding periods x hybrids	18.15**	2.84	2.09

C.V. % Whole Plots = 12.0%
Sub-plots = 15.8%

There were highly significant ($P<0.01$) differences in the insect biomass sampled from each hybrid, and also at each sampling period. The interaction was also highly significant ($P<0.01$)

The effect of feeding period on insect biomass

Table 4.2.10. Mean biomass/plant (mg) of larvae and pupae recovered at weekly intervals, averaged over 3 hybrids

FEEDING PERIOD (DAYS)							
10	17	24	31	38	45	52	59
17.6a[1.]	92.4a	410.9b	1143.0c	1826.5d	1808.9d	1831.2d	1795.7d

[1.] Means followed by the same letter are not significantly different at the 5% level

L.S.D. (5%) = 233.0

Larval biomass increased steadily with time up to 38 days feeding. Thereafter the biomass remained constant due largely to the presence of more pupae than larvae.

The effect of hybrids on insect biomass

There were highly significant differences between the insect biomass removed from hybrids (Table 4.2.11).

Table 4.2.11. Mean insect biomass/plant (mg) of larvae and pupae recovered from 3 hybrids, averaged over 8 sampling dates

HYBRID	INSECT BIOMASS (mg)
F07 x F09	622.2a[1.]
F09 x F08	1181.6b
D50 x K80	1293.4c

[1.] **Means followed by the same letter are not significantly different at the 5% level**

L.S.D. (5%) = 106.6

These differences were influenced predominantly by the differences in larval numbers present in each hybrid (Table 4.2.4). As F07 x F09 had the lowest larval numbers as well as the lowest larval mass of all the hybrids, this hybrid had the lowest biomass. This is also shown in Fig.14 where the increase in biomass in F07 x F09 is expressed as a percentage of the insect biomass recorded from D50 x K80.

The effect of feeding period on insect biomass

Table 4.2.12. Mean larval biomass/plant (mg) of larvae and pupae recovered from 3 hybrids, at weekly intervals

	FEEDING PERIOD (DAYS)							
HYBRID	10	17	24	31	38	45	52	59
F07xF09	6.3a[1.]	31.6c	71.5e	165.7h	512.6l	1287.6p	1470.0t	1432.6x
F09xF08	19.4a	117.8c	532.7f	1323.6i	1888.6m	1917.7q	1768.6t	1884.6y
D50xK80	27.1a	127.7c	628.6f	1939.8j	3078.3n	2221.5r	2254.9u	2069.8y

[1.] Means in columns followed by the same letter are not significantly different at the 5% level

L.S.D. (5%)	Main effect	Interaction
Feeding periods	233.0	301.2
Hybrids	106.6	335.0

There were no significant differences between biomass recorded from each hybrid at the 10 or 17 day samplings (See also Fig.13). By the 24 day sampling F07 x F09 had a significantly ($P<0.05$) lower insect biomass per plant than the other two hybrids. Only at the 31 day sampling did F09 x F08 start showing a significantly lower biomass than D50 x K80. Fig 14 shows the larval and pupal biomass per plant present in F07 x F09, expressed as a percentage of the biomass found in D50 x K80. While larvae were feeding in whorl tissue (10-31 days), larval biomass in D50 x K80 increased at a much faster rate than that of larvae feeding in F07 x F09. This explains the reduction during the period 10-31 days in biomass of larvae feeding in F07 x F09 relative to that of larvae feeding in D50 x K80. As soon as larvae in F07 x F09 commenced stem feeding (31/38 days feeding) they were freed from the effects of the leaf tissue

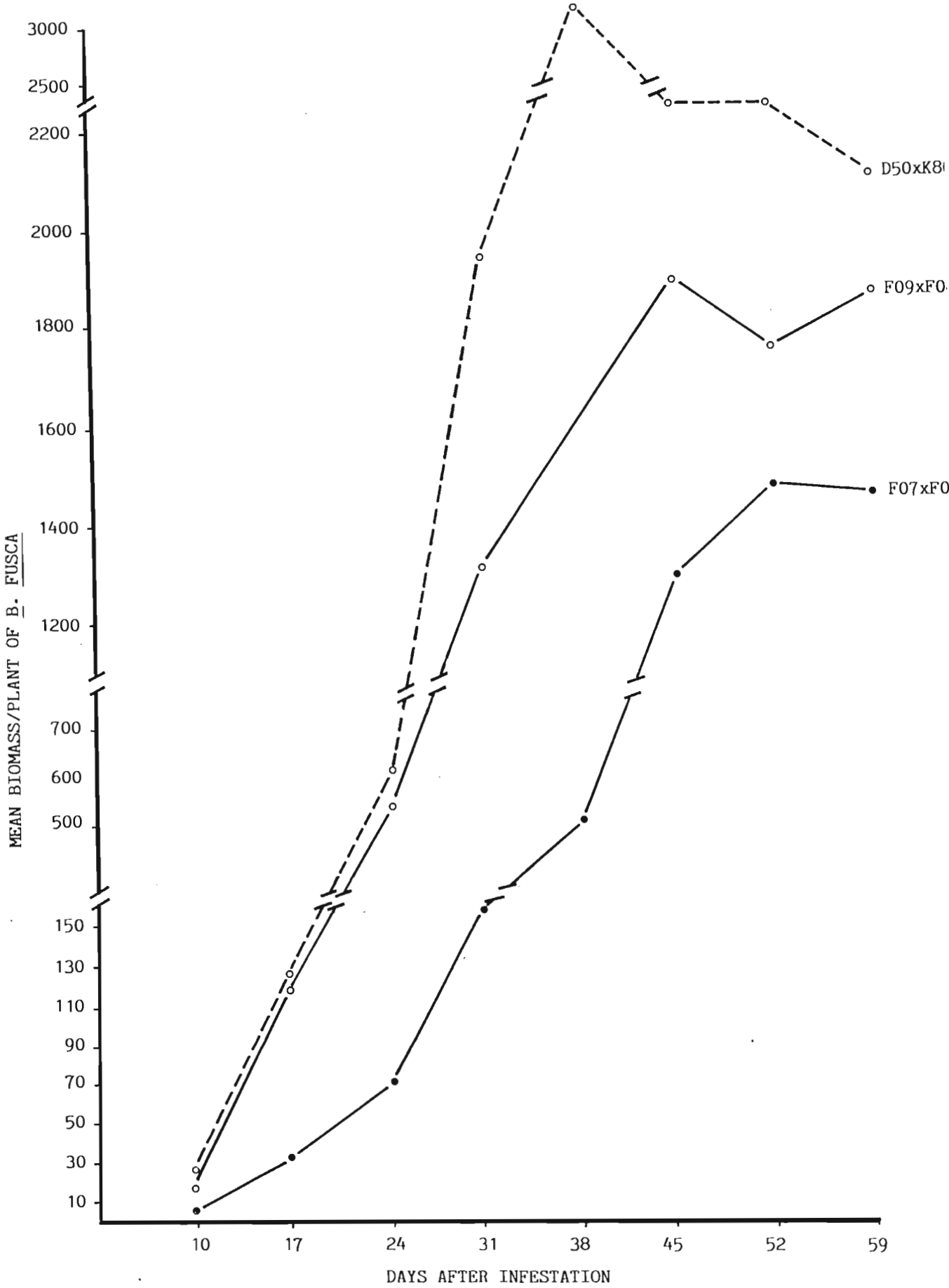


Fig. 13. Mean biomass/plant of B. fusca (larvae and pupae), sampled at weekly intervals from three single cross maize hybrids

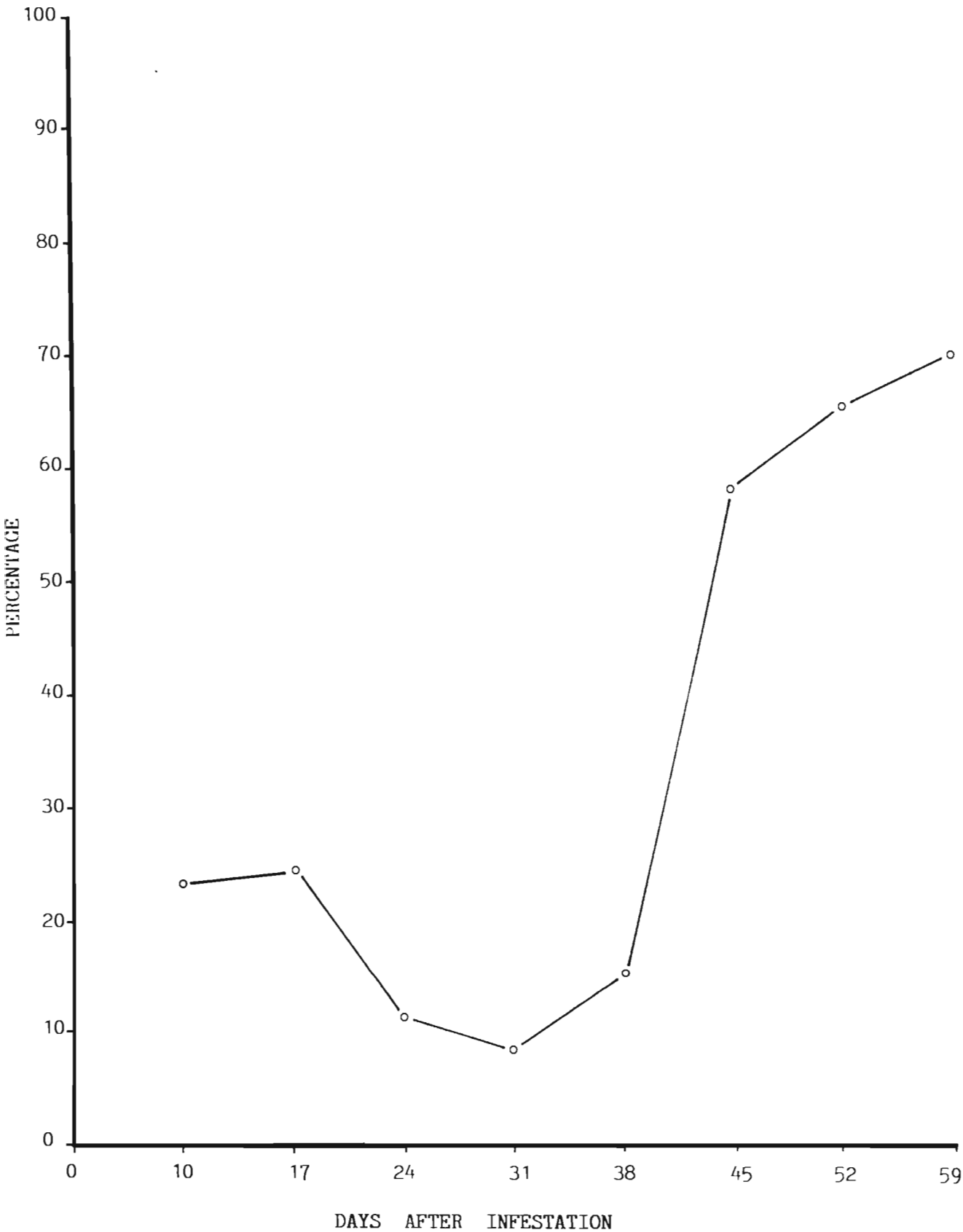


Fig. 14. Larval and pupal biomass/plant in F07 x F09, expressed as a percentage of the biomass in D50 x K80

resistance, and commenced gaining mass at a much quicker rate than before. By the 59 day sampling, biomass in F07 x F09 was nearly 70% of the biomass found in D50 x K80. There was also no significant difference between the biomass found in F09 x F08 and D50 x K80. These data illustrate the importance of the correct timing of larval sampling in assessment of leaf tissue resistance, which should be no earlier than *ca.* 3 weeks feeding, and should be carried out before stem boring occurs.

(d) Stem boring

Larvae of *B. fusca* generally reach the pre-pupal stage while feeding in leaf tissue if the tassel has not yet emerged from the top of the plant. They then move out of the whorl, crawl down the outside of the stem and bore into the stem at any place from just below the tassel to just above ground level. A cavity is excavated, and pupation occurs. No larvae were dissected from plant stems at the 17 day sampling. At the 24 day sampling, however, larvae feeding in F09 x F08 and D50 x K80 had matured sufficiently to commence stem boring prior to pupation (Fig.11). Larvae feeding in F07 x F09 were retarded in their development and only commenced stem boring at the 31 day sampling. At the 31 day sampling, 5.5% of all larvae feeding in F07 x F09 were in stem tissue, compared with 36.2% for D50 x K80, and 38.2% for F09 x F08. By the 38 day sampling the tassels had started emerging from the tops of the plants, forcing all larvae (whether mature or not) to move out of the whorls and to bore into the stems. Once in stem tissue of F07 x F09, larvae feeding was not affected by any resistance mechanism in stem tissue, and larvae gained mass rapidly. The delay in commencement of stem feeding can have a beneficial effect on yield loss. This is because severe damage can occur to stem tissue, resulting in reduced nutrient and water flow to the ear. If this damage can be delayed, it would reduce the extent of damage and field losses would not be so severe. This is discussed fully under 5.2.1. However, the retarded development of larvae feeding on resistant leaf tissue may result in extremely severe stem damage. This is because the larvae can now feed unhindered on stem tissue, and therefore feed for a long

time in order to get to the pre-pupal stage. This extensive stem feeding in "resistant" plants (leaf resistance) can then lead to dramatic yield losses (see 5.2.1).

(e) Pupation

The resistance in the leaf tissue of F07 x F09 retarded larval development. This resulted in pupation commencing later in F07 x F09 than in the other two hybrids (Fig.15). After 38 days feeding approximately 25% of all larvae found in F09 x F08 and D50 x K80 had pupated, but no larvae had pupated yet in F07 x F09. Pupation commenced in F07 x F09 after 38 days feeding. The rate of increase in pupation in all 3 hybrids was similar, with larvae in F07 x F09 pupating about 1.5 weeks later than in the other 2 hybrids.

The pupae removed from each hybrid were similar in mass, and it is evident that leaf resistance coupled with stem susceptibility had no effect on the final mass of pupae:

Table 4.2.13. Mean pupal mass (mg) of pupae found in stem tissue of 3 hybrids after larvae had fed for various periods of time

	Feeding Periods (Days)			
HYBRID	38	45	52	58
F07 x F09	0 pupae	287.4	301.9	299.5
F09 x F08	292.6	299.3	306.1	295.0
D50 x K80	294.5	299.5	305.7	308.9

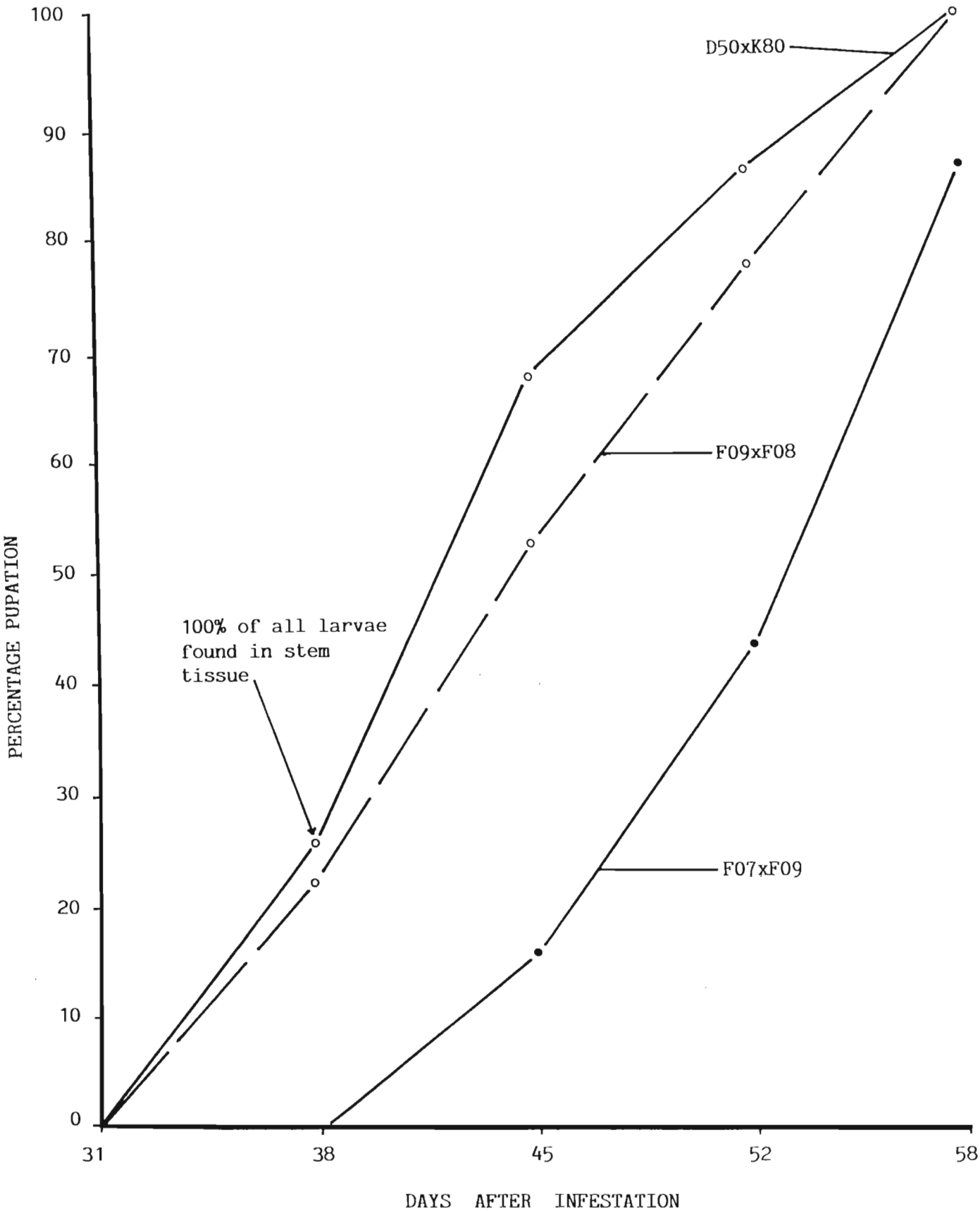


Fig. 15. Rate of pupation of B. fusca in three single cross maize hybrids, expressed as a percentage of B. fusca in the pupal stage

4.2.2 Development of moth and egg stages

Two of Dahms' (1972) criteria used to evaluate resistance were the determination of the number of eggs oviposited, and the reproduction potential of insects that had fed on resistant plants. As was seen in 4.2.1, larvae feeding in plants that contained leaf resistant factors were reduced in number and had their development severely retarded. This had the effect of delaying pupation by between 1 and 2 weeks, which in turn resulted in delayed stem boring. The earlier stem boring occurs, the greater the yield loss (see 5.2.1). Although the larvae feeding in stem tissue of different cultivars eventually all weighed the same, the value of resistant leaf tissue was in delayed stem boring which resulted in reduced stem damage.

The phenology of *B. fusca* does not end with pupation of the first generation. The second generation can cause extensive damage to late planted maize. Although the effects on mass of larvae due to leaf resistance were not evident by pupation it was not known whether any physiological effect was carried through to the adult stage.

The objective of this experiment was therefore to investigate the development of the moth and egg stages of *B. fusca*, after larvae had fed on either resistant or susceptible germplasm.

(i) Materials and methods

The same three single cross hybrids as in 4.1.1.5 were used in this experiment. In addition, another resistant hybrid (D57 x D54) was included. The experiment was planted at the same time as 4.1.1.5 as a randomized complete block, with split plots and four replications. The whole plot treatments (one row of 10 plants per inbred) were four sampling dates for the recording of larval tunneling (entrance holes in the stems were counted after 20, 27, 34 and 41 days feeding). The split plots were the 4 hybrids.

After 55 days feeding in both whorl and stem tissue, by which

time most larvae had pupated in the stems, all the plants were removed from the field. The stems were split, and all the larvae and pupae removed. Data were recorded on numbers and mass of pupae. The pupae were stored under conditions described in 3.1. Dates of moth emergence, moth sex ratio, and the mass of eggs oviposited/female were recorded.

For oviposition studies, moths were placed in pairs (one male and one female) in oviposition containers as described in 3.1.. The eggs were removed and weighed daily. They were placed in sealed 51 cardboard containers which had a high relative humidity maintained by vials of water-soaked cotton wool. Larval eclosion from these eggs was also monitored.

(ii) Results and discussion

(a) Stem entry by larvae

Stem boring was assessed by counting entry holes on the outside of the stems. These were caused by larvae moving into the stems to feed and pupate (Table 4.2.14). Highly significant differences ($P<0.01$) were evident between the number of holes recorded at each sampling date. There were also highly significant ($P<0.01$) differences between the number of holes recorded from each hybrid. The interaction between the two variates was not significant.

Table 4.2.14. Significance of the mean numbers of entrance holes/stem recorded in 4 hybrids, after larvae had fed in whorl tissue for 20, 27, 34 and 41 days

SOURCE OF VARIATION	F	F distribution values	
		1%	5%
Feeding periods	68.75**	6.99	3.86
Hybrids	34.70**	4.41	2.87
Feeding periods x hybrids	0.85 N.S.	2.90	2.15

C.V.% Whole Plots = 14.7%
Sub-plots = 22.4%

The effect of feeding periods on numbers of entrance holes

There were highly significant differences evident between the number of entrance holes in the stems at each sampling date.

Table 4.2.15. Mean numbers of entrance holes/stem recorded in 4 hybrids after 4 feeding periods

	FEEDING PERIOD (DAYS)			
	20	27[2.]	34	41
	0.89a[1.]	2.57b	5.08c	5.27c
% of final count:	16.9%	48.7%	96.4%	100.0%

[1.] **Means followed by the same letter are not significantly different at the 5% level**

L.S.D. (5%) = 0.97

[2.] **Tassels emerged, and all larvae ceased feeding in the whorl tissue.**

Stem boring commenced just before the 20 day sampling, and increased steadily up to the 34 day sampling. After the 34 day sampling, only 3.6% of the larvae had not yet entered the stems. No more holes were recorded after the 41 day sampling.

The time taken for larvae to develop to the pre-pupal stage (which is usually the instar that bores into the stems) varied greatly. Some larvae bored in after only 20 days' feeding, while others entered the stems as late as just before the 41 day sampling.

The effect of hybrids on numbers of entrance holes

There were highly significant ($P<0.01$) differences between the numbers of holes found in the stems of the hybrids.

Table 4.2.16. Mean numbers of entrance holes/stem recorded in 4 hybrids averaged over 4 feeding intervals

HYBRIDS			
D57 x D54	F07 x F09	F09 x F08	D50 x K80
2.39a[1.]	2.61a	4.07b	4.75c

[1.] **Means followed by the same letter are not significantly different at the 5% level**

L.S.D. (5%) = 0.65

These significant differences can be explained as follows:

(i) Stem boring started earlier in F09 x F08 and D50 x K80 than in the other hybrids, so their means for the early sampling periods were higher. These two hybrids lack the resistant factor which delays larval development. Larvae feeding in the whorl tissue therefore developed more rapidly to the prepupal stage than larvae feeding in D57 x D54 and F07 x F09.

(ii) The resistant factor affecting larval numbers reduced the numbers of larvae feeding in D57 x D54 and F07 x F09. This reduced the number of entry holes bored into the stems of these two hybrids.

The effects of these two resistant factors on numbers and development are probably compounded, and are not clear cut.

The effect of feeding periods x hybrids interaction on entrance holes

Table 4.2.17. Mean numbers of entrance holes/stem recorded in 4 hybrids after 4 feeding periods. The percentage of the final count for each hybrid after 41 days is shown alongside in brackets

	FEEDING PERIOD			
HYBRID	20 days	27 days	34 days	41 days
D57xD54	0.20 (4.8)a	1.25(28.6)d	3.87(90.5)h	4.22(100.0)l
F07xF09	0.12 (2.2)a	1.30(28.3)d	4.37(93.5)hi	4.65(100.0)l
F09xF08	1.40(24.1)b	3.20(55.2)e	5.82(99.1)ij	5.87(100.0)m
D50xK80	1.85(28.6)b	4.55(71.4)f	6.27(99.2)j	6.32(100.0)m

1. Means in columns followed by the same letter are not significantly different at the 5% level

L.S.D. 5%	Main effect	Interaction
Feeding periods	0.97	1.20
Hybrids	0.6	1.11

These data are also shown in Fig. 16, and confirm conclusions reached in 4.2.1. The resistant factor in D57 x D54 and F07 x F099 retarded larval development to such an extent that there was a significant delay in stem boring in these 2 hybrids at the 20 day recording. In contrast, already about 25% of all holes recorded over the entire experiment for F09 x F08 and D50 x K80 were already evident. At the 27 day recording, stem boring activity in D57 x D54 and F07 x F09 was still less than that recorded in F09 x F08 and D50 x K80 at the 20 day recording (Table 4.2.17). Just over 70% of the total number of holes in D50 x K80 were evident compared with only about 30% of the holes in D57 x D54 and F07 x F09.

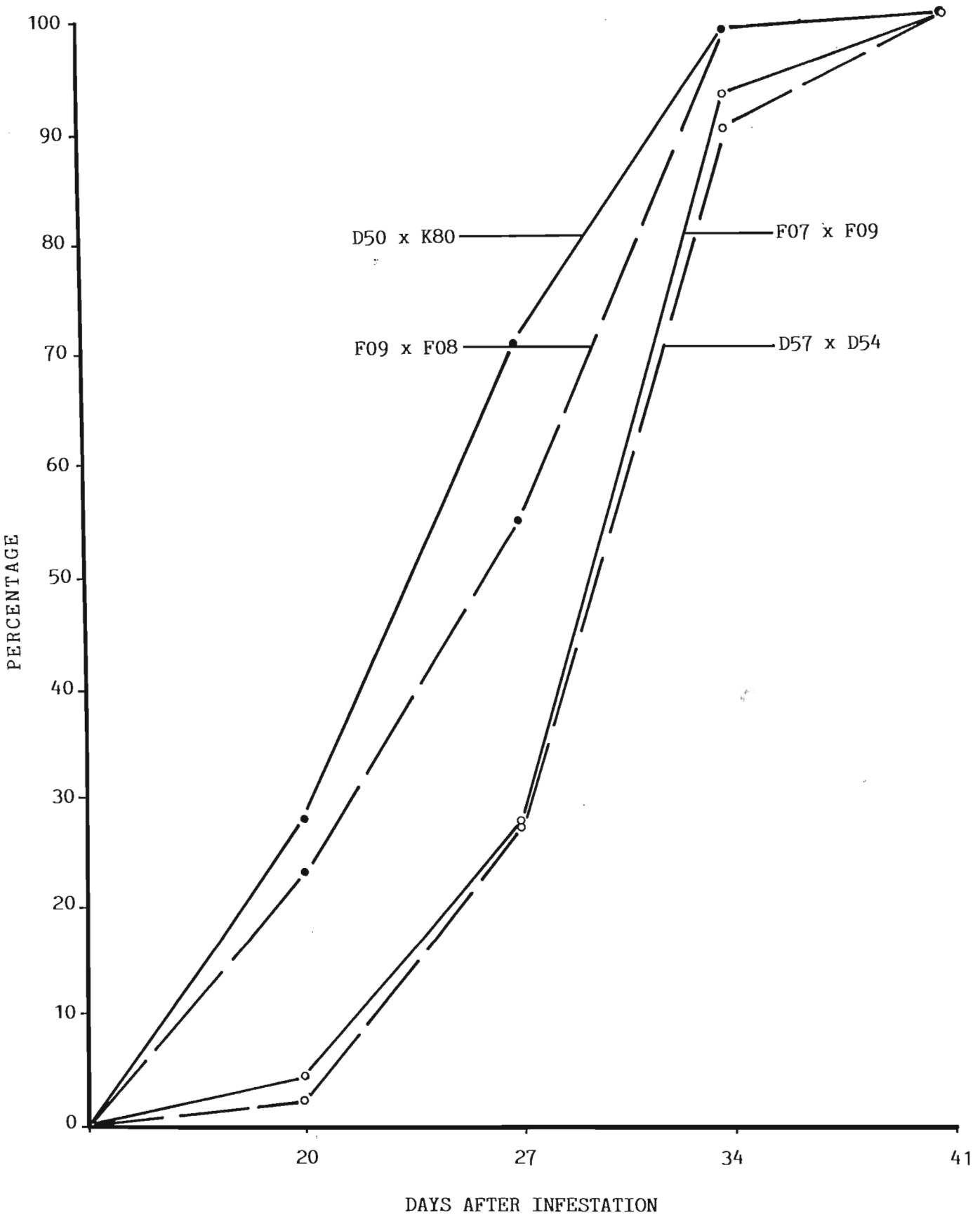


Fig. 16. Mean cumulative number of B. fusca entrance holes per plant stem, expressed as a percentage of the final count after 41 days feeding.

By the 34 day sampling, the number of holes in the two resistant hybrids was still significantly lower than the number of holes in the susceptible hybrids. By the 41 day sampling, as already explained, all larvae were feeding in stem tissue. The final 41 day sampling showed significant differences in the numbers of holes caused by the resistance in the two hybrids reducing larval numbers.

(b) Sampling life stages

After 55 days' feeding, stems were split, and the total number and mean mass of larvae and pupae were recorded.

Numbers of larvae

Table 4.2.18. Significance of the mean numbers of larvae/plant recovered from 4 hybrids after 55 days feeding

SOURCE OF VARIATION	F	F distribution values	
		1%	5%
Hybrids	7.90**	6.99	3.86

C.V. % Plots = 15.9%

There were highly significant differences ($P<0.01$) between the numbers of larvae/plant removed from each hybrid after 55 days feeding.

Table 4.2.19. Mean numbers of larvae/plant recovered from 4 hybrids after 55 days feeding

HYBRID	LARVAE/PLANT	% OF TOTAL NUMBER OF LARVAE & PUPAE
D50 x K80	0.42a[1]	5.9
F09 x F08	0.47a	7.4
D57 x D54	0.75b	21.0
F07 x F09	0.90b	24.3

[1] Means followed by the same letter are not significantly different at the 5% level

L.S.D. (5%) = 0.27

The differences in numbers of larvae/plant and the differences in the larva : pupa ratio are indicative of the resistant factors present in F07 x F09 and D57 x D54. This factor delayed larval development and pupation resulting in a higher larva : pupa ratio in the resistant hybrids than in the susceptible hybrids. Pupal development began earlier in F09 x F08 and D50 x K80 as explained below.

Numbers of Pupae

Table 4.2.20. Significance of the mean numbers of pupae/plant recovered from 4 hybrids after 55 days feeding

SOURCE OF VARIATION	F	F distribution values	
		1%	5%
Hybrids	23.10**	6.99	3.86

C.V.% Plots = 18.7%

There were highly significant ($P<0.01$) differences between the numbers of pupae/plant removed from the 4 hybrids.

Table 4.2.21. Mean numbers of pupae/plant removed from 4 hybrids after 55 days feeding

HYBRID	PUPAE/PLANT	% OF FINAL NUMBER OF LARVAE & PUPAE
F07 x F09	2.80 a[1.]	75.7
D57 x D54	2.82 a	79.0
F09 x F08	5.85 b	92.6
D50 x K80	6.67 b	94.1

[1.] Means followed by the same letter are not significantly different at the 5% level

L.S.D. (5%) = 1.62

The effect of the resistant factor which delayed pupation is very evident. Only 75% of all larvae had pupated in F07 x F09 compared with nearly 95% in D50 x K80. Significantly more pupae were found in the two susceptible hybrids than in the resistant hybrids. This is discussed further under total insect numbers.

Total insect numbers

Table 4.2.22 summarizes the results of the Analysis of Variance, comparing the mean numbers of all insect stages (larvae and pupae)/plant recorded from each hybrid.

Table 4.2.22. Significance of the mean numbers of larvae and pupae/plant recovered from 4 hybrids after 55 days feeding

SOURCE OF VARIATION	F	F distribution values	
		1%	5%
Hybrids	18.22**	6.99	3.86

C.V.% Plots = 16.3%

There were highly significant ($P<0.01$) differences between the numbers of larvae and pupae/plant removed from the 4 hybrids.

Table 4.2.23. Mean numbers of larvae and pupae/plant recovered from 4 hybrids after 55 days feeding

HYBRIDS	LARVAE & PUPAE/PLANT
D57 x D54	3.57 a[1.]
F07 x F09	3.70 a
F09 x F08	6.32 b
D50 x K80	7.09 b

[1.] Means followed by the same letter are not significantly different at the 5% level

L.S.D. (5%) = 1.50

The effect of the resistant factor reducing larval numbers is evident in D57 x D54 and F07 x F09. These hybrids had about half the number of larvae and pupae recorded from the other two hybrids.

The benefit to the farmer (apart from reduced damage) would be a reduction in the number of moths emerging from a resistant hybrid. Oviposition by these moths in late planted maize constitutes the start of the second generation of *B. fusca*. If uncontrolled, this generation can cause severe damage to the stems and ears, resulting in substantial financial losses.

Larval mass

Table 4.2.24 summarizes the results of the Analysis of Variance, comparing the mean mass/larva recorded from each hybrid.

Table 4.2.24. Significance of the mean larval mass of larvae recovered from 4 hybrids after 55 days feeding

SOURCE OF VARIATION	F	F distribution values	
		1%	5%
Hybrids	1.31 N.S.	6.99	3.86
C.V.% Plots	= 2.1%		

When larvae ceased feeding in the leaf tissue, they bored into the stems, and fed on stem tissue. As has been discussed, hybrids possessing leaf resistant factors were found to have susceptible stem tissue. Larvae feeding in these hybrids fed on susceptible stem tissue, and rapidly gained mass. These larvae eventually weighed the same as larvae feeding in the susceptible hybrids, and there were no significant differences in final larval mass:

F07 x F09 = 305.4 mg/larva
F09 x F08 = 303.4 " "
D50 x K80 = 302.0 " "
D57 x D54 = 296.8 " "

Pupal mass

Table 4.2.25 summarizes the results of the Analysis of Variance, comparing the mean mass/pupa from each hybrid.

Table 4.2.25. Significance of the mean pupal mass of pupae recovered from 4 hybrids after 55 days larval feeding

SOURCE OF VARIATION	F	F Distribution values	
		1%	5%
Hybrids	0.27 N.S.	6.99	3.86

C.V. % Plots = 3.9%

All the larvae fed in stem tissue prior to pupation and reached similar mass prior to pupation. Any leaf resistance had therefore become ineffective by the 55 day sampling date. No significant differences were evident between pupal mass in the different hybrids:

F09 x F08 = 307.2 mg/pupa
D57 x D54 = 305.2 " "
D50 x K80 = 301.6 " "
F07 x F09 = 300.6 " "

Female pupae were considerably heavier than male pupae for all hybrids. There were no significant differences between the pupal mass of pupae of the same sexes in the hybrids.

Table 4.2.26. Mean pupal mass (mg) of male and female pupae recovered from 4 hybrids after 55 days feeding

HYBRID	MALE	FEMALE
F09 x F08	250.9	353.4
D57 x D54	252.7	357.6
D50 x K80	249.6	353.6
F07 x F09	249.0	352.2

Total insect mass

Table 4.2.27 summarizes the results of the Analysis of Variance, comparing the mean mass of all insects (larvae and pupae) found in each hybrid.

Table 4.2.27. Significance of the mean insect mass of larvae and pupae recovered from 4 hybrids after 55 days feeding

SOURCE OF VARIATION	F	F Distribution values	
		1%	5%
Hybrids	0.39 N.S.	6.99	3.86

C.V.% Plots = 4.1%

Non significance was due to there being no significant differences between larval and pupal mass in the different hybrids after 55 days feeding. The following mean insect masses were recorded:

D50 x K80 = 310.9 mg/insect
F09 x F08 = 307.1 " "
D57 x D54 = 303.6 " "
F07 x F09 = 302.2 " "

Larval biomass

Table 4.2.28 summarizes the results of the Analysis of Variance, comparing the mean larval biomass/plant found in each hybrid.

Table 4.2.28. Significance of the mean larval biomass/plant recovered from 4 hybrids after 55 days feeding

SOURCE OF VARIATION	F.	F Distribution values	
		1%	5%
Hybrids	7.60**	6.99	3.86

C.V.% Plots = 16.9%

Although the mean larval mass was similar in all hybrids after 55 days feeding, the mean numbers of larvae/plant varied significantly between hybrids. This resulted in highly significant differences in larval biomass (mg/plant) present in the hybrids:

D50 x K80 = 128.4a[1.]
F09 x F08 = 144.5a
D57 x D54 = 223.8b
F07 x F09 = 275.6b

[1.] Means followed by the same letter are not significantly different at the 5% level

L.S.D. (5%) = 79.8

Pupal biomass

Table 4.2.29 summarizes the results of the Analysis of Variance, comparing the mean pupal biomass/plant found in each hybrid.

Table 4.2.29. Significance of the mean pupal biomass/plant recovered from 4 hybrids after 55 days larval feeding

SOURCE OF VARIATION	F	F distribution values	
		1%	5%
Hybrids	21.96**	6.99	3.86

C.V. % Plots = 12.3%

There was a significantly lower pupal biomass (mg/plant) in the two resistant hybrids:

F05	x F07	=	843.8a[1]
D57	x D54	=	860.4a
F09	x F08	=	1796.7b
D50	x K80	=	2014.7b

1. Means followed by the same letter are not significantly different at the 5% level

L.S.D. (5%) = 504.9

Mean insect biomass

Table 4.2.30 summarizes the results of the Analysis of Variance, comparing the mean insect biomass/plant found in each hybrid.

Table 4.2.30. Significance of the mean insect biomass/plant recovered from 4 hybrids after 55 days feeding

SOURCE OF VARIATION	F	F distribution values	
		1%	5%
Hybrids	**18.14****	**6.99**	**3.86**

C.V. % Plots = 11.4%

There were highly significant differences evident between the mean insect biomass (mg/plant) found in each hybrid:

D57	x D54	=	1084.9a[1.]
F05	x F07	=	1119.2a
F09	x F08	=	1941.9b
D50	x K80	=	2142.2b

[1.] Means followed by the same letter are not significantly different at the 5% level

L.S.D. (5%) = 467.4

As will be discussed in Chapter 5, a reduced insect biomass usually results in less stem damage, and therefore less yield reduction.

A summary of these data are shown in Table 4.2.31.

Table 4.2.31. Mean numbers, mass and biomass of larvae and pupae per plant recovered from 4 hybrids after 55 days feeding

	HYBRID			
LARVAE	D57 x D54	F07 x F09	F09 x F08	D50 x K80
Mean no. larvae/plant:	0.75b[1.]	0.90b	0.47a	0.42a
Mean mass (mg) /larva	: 296.8 m	305.4 m	303.4 m	302.0 m
Mean larval biomass/ plant	: 223.8 x	275.6 x	144.5 y	128.4 y
PUPAE	D57 x D54	F07 x F09	F09 x F08	D50 x K80
Mean no. pupae/plant	: 2.82a	2.80a	5.85b	6.67b
Mean mass (mg) /pupa	: 305.2 m	300.6 m	307.2 m	301.6 m
Mean pupal biomass /plant	: 861.0 x	843.8 x	1796.7 y	2014.7 y
LARVAE & PUPAE	D57 x D54	F07 x F09	F09 x F08	D50 x K80
Mean no./plant	: 3.57a	3.70a	6.32b	7.09b
Mean mass (mg)	: 303.6 m	302.2 m	307.1 m	310.9 m
Mean biomass/plant	: 1084.9 x	1119.2 x	1941.9 y	2142.2 y

[1.] Means in the same row with the same letters are not significantly different at the 5% level

Moth emergence and fecundity

In both 4.2.1 and this experiment, larvae commenced pupating later in resistant hybrids than in the susceptible hybrids.

Moth emergence in all hybrids followed a similar trend (Fig.17). At 50% moth emergence, there was about a 4 day delay in moth emergence in the resistant hybrids, compared with moth emergence in the susceptible hybrids. This phenomenon is due to the delay in larval development in the resistant hybrids. Under field conditions, this delay would be of doubtful value in such hybrids under natural infestation.

The sexes of the moths were recorded, and there was no significant difference in the ratio of male : female between hybrids:

1:1,2 for D57 x D54
1:1,3 for F07 x F09
1:1,0 for F09 x F08
1:1,1 for D50 x K80

Investigation of moth fecundity showed that it was unaffected by resistant factors acting on larvae feeding in resistant plants (Table 4.2.32.)

Table 4.2.32. Oviposition data of moths recovered from pupae having developed in 4 hybrids

HYBRID	NUMBER MATING PAIRS	MEAN MASS (mg) OF EGGS PER FEMALE MOTH	PERCENTAGE HATCH OF EGGS
D57 x D54	70	5.97	98.7
F07 x F09	68	6.02	100.0
F09 x F08	160	5.49	100.0
D50 x K80	176	5.99	99.4

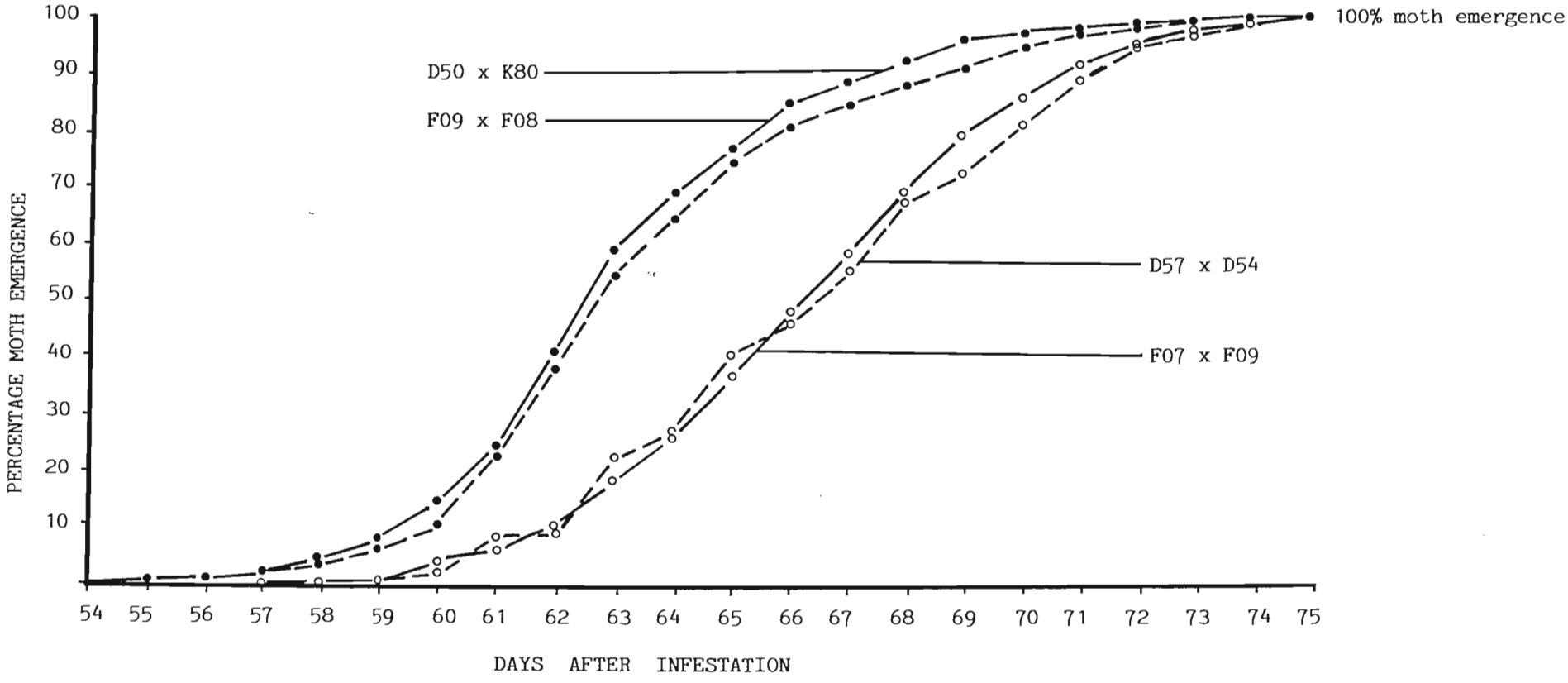


Fig. 17. Cumulative percentage moth emergence, expressed as a percentage of all moths emerged for each hybrid

In conclusion, resistant leaf factors which delayed larval development resulted in a delay in stem boring. The later in the plant growth stage that stem boring commences the less the damage. This phenomenon therefore is of value in an HPR programme, and has a practical use in the field. This will be discussed fully in Chapter 5.

The delay in pupation and therefore of moth emergence is of negligible value. During the period of second generation moth flight which extends over a 6-7 week period from late January to mid March, a 4 day delay in moth emergence is of no consequence.

Of the maize genotypes studied so far, all had susceptible stem tissue. This resulted in rapid mass gains of growth-retarded larvae once they had moved out of the whorl and fed on stem tissue. The desired result of undersize pupae and possibly of lowered moth viability and fecundity was not observed. If stem resistance and leaf resistance were present in a single genotype, then this desired result might be obtained.

Stem damage is discussed in Chapter 5, and there are certain maize genotypes which show a more resistant reaction than the genotypes investigated above. As will be explained, there is often an interaction of so many factors, that a resistant cultivar (leaf reaction) can actually show a greater yield loss than a susceptible cultivar.

4.3. TASSEL RESISTANCE : DIFFERENCES IN LEVELS OF RESISTANCE BETWEEN VARIOUS TASSEL TISSUES OF SEVERAL MAIZE GENOTYPES

Larvae feeding in the plant whorl will continue feeding therein until the pre-pupal stage or until they are forced out by the emerging tassel. If they have not yet reached the pre-pupal stage when they penetrate the developing tassel, they will continue feeding on the tassel tissue, especially within its stem, which often results in the death of the tassel (Plate 16). This feeding continues until tassel emergence from the top of the plant. This period of tassel feeding may last from 1 day up to two weeks. Depending on the age of the plants at infestation, the ages of larvae at tassel emergence may also vary considerably - the older the plants are when infestation occurs, the younger the larvae will be at tassel emergence.

It is thus evident that the ages of larvae and the period of tassel feeding may vary considerably from field to field on a commercial maize farm. It has often been observed in the field that larvae that were feeding in tassel tissue were left exposed on the tassels, without penetrating the tissue, when the tassels emerged. This behaviour is contrary to the normal larval migration down and into the stem which occurs after tassel emergence. These larvae were seen to be moribund and obviously adversely affected in some way. They were thus exposed to adverse weather conditions, parasitism and predation, and were obviously incapable of fending for themselves and of developing further. It is possible that the tassels had a resistant effect on the larvae.

It is apparent from previously mentioned data and below (see Chapter 5) that there are different resistance mechanisms present in leaf and stem tissue. Although the larvae generally spend several days feeding in tassel tissue, it was thought to be an important area of investigation. The levels of resistance in tassel tissue of several maize genotypes were therefore compared with resistance in leaf tissue.

Plate 16. Susceptible tassel in genotype with excellent leaf resistance.

Larvae feed predominantly on the glumes, often moving from the rolled up glumes into the tassel stem. They also feed on the tassel peduncle (by which the glumes (containing pollen) are attached to the stem). They thus have three feeding sites, which were separately screened for resistance in this experiment.

(i) Materials and methods

As all the tassels were required simultaneously, ten of Pioneer's range of elite inbreds were selected for their similarity in flowering times.

The seeds were planted on 1st November 1983, and the plants received no artificial or natural infestation. Just prior to tassel emergence from the whorl, the tassels were removed from the plants and brought into the laboratory.

The tassels of each inbred were divided up into stems, peduncles and glumes and approximately 20g of each placed in petri dishes. The experiment was replicated three times. The dishes were kept in a totally dark room at *ca.* 27 °C and ambient humidity. Batches of twenty 15-day old larvae, removed after 15 days feeding in a highly susceptible inbred, were weighed and the total mass and mean mass recorded. They were then placed on the plant parts in the petri dishes. Each inbred therefore had 60 larvae (3 replicates x 20 larvae) per tassel treatment. The larvae were allowed to feed for 8 days, and were then re-weighed. No mortality occurred. To determine whether repellence or antibiosis was the resistance mechanism involved, the activity of the larvae was observed as to whether they had settled and were feeding or were moving around.

The 20 larvae/treatment were sorted out into glass vials prior to application on the tassel parts. It therefore took only a few minutes to place them in the petri dishes containing the food source. They were then allowed to settle in a darkened room for about one hour. The fluorescent light was then switched on, and the number of larvae seen moving around (as opposed to larvae

settled and feeding) was recorded for each treatment.

(ii) Results and discussion

In the following analyses, where appropriate, the Analysis of Covariance (ANCOV) was used to adjust for random variation in the initial masses of the larvae for the various cultivars. As indicated earlier, due to heterogeneity of variance, the log transformation was used to stabilize this variance.

(a) Larval mass

The Analysis of Variance showed highly significant differences between the increase in mass of larvae feeding on the inbreds. There were also highly significant differences evident between the amounts of resistance expressed by the various tassel parts. The interaction was also highly significant.

Table 4.3.1. Significance of the mean larval mass of larvae recovered after 8 days feeding on tassel tissue of 10 inbreds

SOURCE OF VARIATION	F	F Distribution Values	
		5%	1%
Cultivars	41.79**	2.41	3.94
Feeding site	26.42**	3.59	6.11
Cultivars x feeding site	17.55**	1.62	2.01

C.V.% Whole Plots = 3.7%
Sub-plots = 5.9%

The effect of inbreds on larval mass gain

Table 4.3.2. Mean mass (mg) of larvae* before and after 8 days feeding on tassel tissue from 10 inbreds

*15 days old at commencement of experiment

	MEAN MASS (mg)			ACTUAL
INBRED	INITIAL	FINAL	% INCREASE	INCREASE (mg)
M52	14.5	25.5a[1]	75.8	11.0
M50	15.4	28.5a	85.1	13.1
K11	14.6	30.3ab	107.5	15.7
J33	14.8	32.3bc	117.6	17.5
J22	14.9	35.9c	140.9	21.0
J34	15.2	46.6d	221.1	31.4
D50	14.7	62.2e	323.1	47.5
J26	14.7	75.3f	412.2	60.6
F23	15.0	80.8g	438.7	65.8
F10	14.7	81.9g	457.1	67.2

[1]Means followed by the same letter are not significantly different at the 5% level. Based on log transformation and adjustment for covariance.

Larvae feeding on the different inbreds showed significantly varying increases in mass gain. As the larvae did not all have an identical initial mass (although these differences were not significant), the percentage mass gain was also utilized as a

measure of resistance affecting growth. The increases ranged from 11,0mg (75.8% increase) for larvae feeding on M52 to 67,2mg (457.1% increase) for larvae feeding on F10, illustrating a great range in resistant factors present in the various inbreds.

The effect of different tassel tissues on larval mass gain

Table 4.3.3. Mean mass (mg) of larvae before and after 8 days feeding on various tassel parts, averaged over 10 inbreds (mean increase in brackets)

TASSEL STEM			TASSEL PEDUNCLE			TASSEL GLUMES		
Initial	Final	% inc.	Initial	Final	% inc.	Initial	Final	% inc.
14.8	26.5	79.0	25.0	96.8	287.2	14.8	26.5	79.0

There was a 287.2% mass increase when larvae fed on the peduncles, compared with a mean increase of only 79.0% when larvae fed on either the stem tissue or glumes. The peduncles obviously offer a far more nutritious food source than either the stems or glumes, which may contain resistance factors reducing larval mass gain.

The effect of inbred x feeding site on larval mass gain

Table 4.3.4. Mean mass (mg) of larvae before and after 8 days feeding on various tassel parts from 10 inbreds

INBRED	TASSEL STEM			TASSEL PEDUNCLE			TASSEL GLUMES		
	INITIAL	FINAL	INCR.	INITIAL	FINAL	INCR.	INITIAL	FINAL	INCR.
M52	15.0	20.8a[1]	5.8	14.9	28.2a	13.3	13.7	27.6cd	13.9
M50	15.4	23.0ab	7.6	15.8	32.2a	16.4	14.9	30.3de	15.4
K11	14.2	24.7b	10.5	14.3	45.6a	31.3	15.2	20.6a	5.4
J33	14.6	24.4b	9.8	15.5	46.8a	31.3	14.3	25.5bc	11.2
J22	14.4	25.7b	11.3	15.2	50.8a	35.6	15.2	31.2e	16.0
J34	15.0	27.4bc	12.4	14.6	88.7b	74.1	15.9	23.6ab	7.7
D50	14.4	29.0cd	14.6	14.7	137.0c	122.3	14.9	20.5a	5.6
F23	15.5	31.3d	15.8	15.2	173.9d	58.7	14.4	37.4f	23.0
J26	14.9	29.4d	14.5	14.4	170.8d	156.4	14.9	25.6bc	10.7
F10	14.6	29.5d	14.9	15.1	193.8d	178.7	14.6	22.5ab	7.9

[1]Means in each column followed by the same letter are not significantly different at the 5% level
Tassel L.S.D. = 3.9; Peduncle L.S.D. = 24.9; Glumes L.S.D.= 4.1

Table 4.3.5. Mean percentage increase in larval mass after 8 days feeding on various tassel parts

INBRED	TASSEL STEM	TASSEL PEDUNCLE	TASSEL GLUMES
M52	38.7	89.3	101.4
M50	49.3	103.8	103.3
K11	73.9	218.9	35.5
J33	67.1	201.9	78.3
J22	78.5	234.2	105.3
J34	82.6	507.5	48.4
D50	101.4	831.9	37.5
J26	97.3	1086.1	159.7
F23	101.9	1044.0	71.8
F10	102.0	1183.4	54.1

There was no obvious pattern in the levels of resistance recorded between the various tassel tissues of each inbred. Some inbreds showed more resistance in the tassel stem (M52, M50, J33, J22, J26)than in other parts. Others had more resistance in the glumes (K11, J34, D50, F23, F10). On none of the inbreds did the

peduncles show more resistance than the stem or glume tissue. The smallest range in percentage increase in larval mass between parts of the same inbred tassel occurred in M52 (38,7% to 101,4%), and the largest range occurred in F10 (54,1% to 1183,4%).

The greatest range of resistance was measured in the tassel peduncles. The lowest percentage mass gain was recorded in M52 (89,3%) compared with the most susceptible inbred F10, which had an increase in larval mass of 1183,4%. Both tassel stem tissue and tassel glume tissue showed smaller ranges of 38,7% to 102,0% and 35,5% to 159,7% respectively. These widely differing ranges illustrate wide diversity in the levels of resistant factors present between the different inbreds, and especially between the different parts of any one tassel.

(b) Larval migration

The recording of larval migration was not a precise measurement. Within the same treatment some larvae were totally stationary while feeding, some migrated only for a short time before settling down, and others moved continually. The amount of migration over a 5 minute period was therefore rated visually on a 0 to 5 scale (0 = no migration; 5 = continual migration).

Table 4.3.6. Larval migration on various types of tassel tissue, rated on a 0 to 5 scale (0 = no migration, 5 = continual migration) (% mass gain alongside in brackets - see Table 4.3.5.)

INBRED	TASSEL STEM	TASSEL PEDUNCLE	TASSEL GLUMES
M52	5 (5.8)	4 (13.3)	0 (13.9)
M50	1 (7.6)	1 (16.4)	0 (15.4)
K11	3 (10.5)	3 (31.3)	0 (5.4)
J33	3 (9.8)	2 (31.3)	0 (11.2)
J22	3 (11.3)	2 (35.6)	0 (16.0)
J34	2 (12.4)	0 (74.1)	4 (7.7)
D50	1 (14.6)	0 (122.3)	5 (5.6)
F23	1 (15.8)	0 (158.7)	0 (23.0)
J26	1 (14.5)	0 (156.4)	0 (10.7)
F10	0 (14.9)	0 (178.7)	2 (7.9)

The results are so varied as to warrant individual comment on the mass gain in each tassel part for each inbred (see Table 4.3.7). As no larval mortality was observed there was obviously no antibiosis which affected larval numbers (as was found in leaf tissue). The conclusion therefore is that the high ratings for larval migration indicate repellence. Where low mass gain was coupled with low larval migration, antibiosis affecting mass gain is indicated. There were also reactions that were intermediate.

Table 4.3.7. Resistance mechanisms (repellence/antibiosis) and classification of resistance observed in different types of tassel tissue from different maize genotypes. (Based on Table 4.3.6)

	TASSEL STEM		TASSEL PEDUNCLE		TASSEL GLUME	
INBRED	Repell.	Antib.	Repell.	Antib.	Repell.	Antib.
M52	High	Imp.to assess[1]	High	Imp.to assess	Nil	High
M50	Low	High	Low	High	Nil	High
K11	Interm.	Imp. to assess	Interm.	Imp. to assess	Nil	High
J33	Interm.	Imp. to assess	Low	Imp. to assess	Nil	High
J22	Interm.	Imp. to assess	Low	Imp. to assess	Nil	High
J34	Low	High	Nil	Intermed.	High	Imp.to assess
D50	Low	High	Nil	Low	High	Imp.to assess
F23	Low	High	Nil	Low	Nil	High
J26	Low	High	Nil	Low	Nil	High
F10	Nil	High	Nil	Low	Low	High

[1]**Antibiosis impossible to assess as high level of repellence precluded the larvae from feeding for any length of time.**

As the various types of resistance mechanisms show a large range of responses from the larvae, the mechanisms are probably additively inherited.

It is interesting to compare these data with data on mass gain of 15 day old larvae feeding for 10 days on inbred leaf tissue (Table 4.1.21.). Some of the inbreds are common to both experiments (K11, D50, F23). Despite the fact that different base levels of mass are used in the two experiments, the comparisons are of interest.

Table 4.3.8. A comparison (data from Table 4.1.21.) of % mass gain of larvae feeding for 10 days in whorl tissue and 8 days in tassel tissue of 3 inbreds (Table 4.3.5.)

	WHORL TISSUE		TASSEL STEM		TASSEL PEDUNCLE		TASSEL GLUMES	
INBRED	Initial mass(mg)	% mass gain over 10 days	Initial mass(mg)	% mass gain over 8 days	Initial mass(mg)	% mass gain over 8 days	Initial mass(mg)	% mass gain over 8 days
K11	7.1	771.8	14.2	73.9	14.3	218.9	15.2	35.5
D50	3.6	433.6	14.4	101.4	14.7	831.9	14.9	37.5
F23	9.4	780.8	15.5	101.9	15.2	1044.0	14.4	159.7

Larvae feeding in the whorl tissue of these inbreds showed a greater mass gain than larvae feeding in all tassel tissue, except for the peduncle tissue of D50 and F23. Table 4.1.21. shows the lowest percentage mass gain of 356,3% for larvae feeding in whorl tissue of F03, and the highest of 986,0% for larvae feeding in M23. These contrast markedly with the percentage mass gain range of 38,7% to 102,0% for larvae feeding in tassel stem tissue (Table 4.3.5.), showing far higher levels of resistance present in the tassel stem than in whorl tissue. It is also possible that the whorl tissue may simply be more nutritious than tassel tissue. The effect of either phenomenon is however the slower development of larvae.

A comparison of percentage mass gain in tassel peduncle tissue and whorl tissue shows that there was generally more resistance present in the tassel peduncle than in whorl tissue, as only on 3 of the 10 inbreds in Table 4.3.5 did larvae show a greater mass gain than on the 11 inbreds in Table 4.1.21. The resistance in the tassel glumes was in all cases greater than that observed in the whorl tissue of the 11 inbreds.

The relevance of these findings is difficult to ascertain. Larvae can start feeding on tassel tissue when the tassel is deeply enclosed within the whorl. If repellence manifests itself, larvae

would be forced to migrate, presumably out of the whorl and into the stem, where they could cause extensive damage (see 4.1.1.5.). It may therefore not be a desirable type of resistance. However, it would expose the larvae to environmental, parasitoid and predator activity while the larvae were migrating down the stem. Tassel death is not of economic concern, as there will be many other plants in the field shedding pollen to ensure pollination of the damaged plants. Tassel removal has actually been found to be a yield increasing procedure in maize seed production lands where the "female" parent is deliberately detasseled (Farwell[7], *pers. comm).*

Even antibiosis in the tassel is of dubious value. It retards the growth of larvae, resulting in less damage to the tassel (which damage, as explained, is of little value). What does happen is that instead of continued larval feeding in the aerial parts of the plant, larvae will now be retarded in development, and will need to continue feeding to get to the pre-pupal stage. This feeding will then continue in the stem where severe yield loss can occur. Conversely, if no resistance occurs, larvae may rapidly complete their development in leaf tissue and move into stem tissue for pupation only, resulting in very little damage. This is all discussed fully in Chapter 5.

---

[7] A.J. Farwell, Pioneer Seed Company, Box 19, Greytown, 3500, South Africa

5. EFFECT OF *B. FUSCA* ON THE PLANT

Larvae feeding in maize whorl tissue are affected by 3 resistance mechanisms. These mechanisms reduced larval numbers by antibiosis or repellence, or retarded their growth. The resultant varying amounts of larval biomass were the direct cause of different amounts of damage to the plants.

The reactions of maize plants exposed to borer attack have been measured in several ways. Various researchers in the U.S.A. have evaluated insect damage on maize by rating leaf damage, stem tunneling, number of holes per plant, stalks girdled, ear damage, yield loss and stunting (e.g. Davis *et al.*(1979) on the Fall Army Worm; Guthrie *et al.* (1970, 1978, 1980, 1984)) on the European Corn Borer; Starks *et al.* (1982) on the South Western Corn Borer). Of all these methods, leaf damage rating is the quickest and a very reliable field method of damage assessment.

Attempts have been made by several researchers to quantify the extent of damage due to the uncontrolled feeding of larvae in field maize. These investigations have centred around natural infestations. These varied considerably as did the ages of the various crops at the time of their infestation. Yield losses due to *B. fusca* infestations in commercial crops ranged from a 14 % infestation giving a yield loss of 9.8% (Anon 1975) to as high as 75% yield loss (Matthee *et al*, 1971). Walker (1960 a) investigated a 49% infestation with a yield loss of 37% in untreated plots. Swaine (1957) recorded 22% damaged plants in untreated lands, and harvested 83% more grain from uninfested plants than infested plants. As will be discussed later, these varying responses were probably dependent on the amount of time spent by the stalk borer feeding in the stem.

Kuhn (1978) reported the first investigation of resistance to *B. fusca* under artificial infestation. He recorded leaf damage, dead-heart and cob damage caused to 40 inbred lines. He concluded that all three methods of damage assessment were more or less equally effective and comparable in assessing the effect of

B. fusca on the plants. Van Rensburg *et al.* (1988 c) assessed plant damage by rating leaf damage, the number of damaged internodes, length of tunneling in the stalk, tassels damaged and dead-heart. They also concluded that despite high Coefficients of Variation, all criteria were useful in the evaluation of stalk borer damage. Of least use were recordings of the length of stalk tunneling and dead-heart counts. To reduce variation between plants of the same genotype, it is essential in the initial evaluation of the different types of borer damage to utilize homozygous plants, such as inbred lines, or identical plants of single cross hybrids. Van Rensburg's use of heterozygous populations could have contributed to the high C.V.'s recorded in his experiments. Fourie (1984), in addition to leaf damage, dead-heart and ear damage, also rated height reduction between infested and uninfested plants.

A series of artificially infested experiments was carried out by the author and assessment was made of the varying amounts of leaf damage, stem damage, height reduction and yield loss. As the larvae change from feeding on leaf tissue to stem tissue, it is probable that they encounter a totally new set of plant substances, with resistant factors different from those of the leaf tissue. The question then arises: If certain maize genotypes show a resistance reaction to stalk borer feeding in the whorl, is that leaf resistance effective throughout the larval feeding period in both leaf and stem tissue, and are the yields of these maize genotypes affected differently? The following experiments were designed to answer that question, and to investigate the effect of first generation stalk borer on maize plants.

5.1 LEAF DAMAGE

Stalk borer generally infest maize crops when the plants are any age between about 21-35 days post-emergence. Larvae eclose, feed on the egg shells, and then within the next 24 hours, migrate into the plant whorl where they commence feeding. The damage appears about 4-5 days later as small shotholes. As the larvae continue feeding, the holes get larger and more numerous. Eventually they coalesce and cause severe leaf shredding and often the death of the growing point. As leaf damage is a quick and efficient method of evaluating the interaction between plants and larvae, various experiments were carried out to investigate several aspects of leaf feeding and leaf damage rating.

5.1.1 Leaf damage caused by various infestation levels

In order to develop resistant maize germplasm, it is essential to be able to distinguish small differences in damage between genotypes. If the infestation is too severe, it is then difficult to select any of the more resistant plants due to their all being rated as highly susceptible. Conversely, if too light an infestation is applied, all the plants appear resistant, with a similar lack of success in selection. It was therefore necessary to determine the levels of infestation which gave the greatest spread of damage between genotypes. The objective of this experiment was to investigate the most efficient and economical number of larvae to apply to each plant. It is also important in any HPR programme to utilize insect numbers as economically as possible. This experiment was designed to investigate these two parameters.

(i) Materials and methods

Four single cross hybrids were planted on 16th October 1981, in a completely randomized block design with split plots and three replications. The whole plot treatments were the hybrids, and the sub-plot treatments were 10 infestation levels. Each replicate of each hybrid consisted of 1 row of 10 plants.

As larvae migrate out of the whorl just prior to pupation, and may enter the stems of nearby plants to pupate, barrier rows were densely planted between treatment rows in order to prevent larval migration and confusion of the stem damage assessment.

All plants were infested 32 days post emergence with one of the following larval infestation levels: 4.1; 8.7; 14.6; 19.6; 25.3; 31.0; 35.8; 42.7; 44.9; 51.5 larvae/plant (representing desired treatment levels of 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 larvae/plant). Leaf damage was assessed by visually rating the extent of leaf damage on each plant on a 1 to 5 scale after 25 days feeding.

(ii) Results and discussion

There were highly significant ($P<0.01$) differences between the leaf damage ratings recorded from the hybrids. Damage resulting from the different infestation levels also varied significantly ($P<0.01$), as did values recorded for the interaction.

Table 5.1. Significance of mean leaf damage ratings of 4 single cross maize hybrids, infested with 10 larval infestation levels, rated after 25 days feeding

SOURCE OF VARIATION	F	F DISTRIBUTION VALUES	
		5%	1%
Cultivars	346.75**	4.76	9.78
Infestation level	404.57**	2.07	2.72
Cultivar x inf. level	8.53**	1.62	2.01

C.V.% Whole Plots = 2.0%
Sub-plots = 5.3%

The effect of cultivars on leaf damage

The leaf damage ratings are shown in Table 5.2.

Table 5.2. Mean leaf damage ratings after 25 days feeding in 4 single cross hybrids, averaged over 10 infestation levels

CULTIVAR			
F23 x F09	F23 x F07	M23 x D50	M23 x 56
2.34a [1.]	2.82b	3.55c	3.75c

[1.] Means followed by the same letter are not significantly different at the 5% level

L.S.D. (5%) = 0.31

F23 x F09 and F23 x F07 were selected for previously having showed a resistant leaf feeding reaction. The other two cultivars were selected as having showed a susceptible reaction. These prior conclusions were confirmed in these data.

The effect of infestation levels on leaf damage

Table 5.3. Mean leaf damage ratings after 25 days feeding resulting from 10 different infestation levels, meaned over 4 single cross hybrids

INFESTATION LEVEL (larvae/plant)									
4.1	8.7	14.6	19.6	25.3	31.0	35.8	42.7	44.9	51.5
1.63a[1.]	1.92a	2.31b	2.63c	3.05d	3.33d	3.83e	3.96df	4.22f	4.27f

[1.] Means followed by the same letter are not significantly different at the 5% level

L.S.D. (5%) = 0.31

There were significant differences between leaf damage recorded for several of the infestation levels. Generally, the more larvae applied, the higher the damage. The increase was fairly linear up to the 35,8 larval level. At the lower levels, there was a significant difference between the 8.7 and 14.6 level, and between the 14.6 and 19.6 level. There was a significant difference between the 19.6 and 25.3 level, but not between the 25.3 and 31.0 level. After a significant difference between the 31.0 and 35.8 level, there were only slightly significant differences in leaf damage ratings of the infestation levels. The leaf damage ratings were not as severe in this experiment as in Table 4.1.1 (see also Table 5.6). A wider range of values was observed over infestation levels, and therefore more significant differences occurred than were recorded in Table 4.1.1.

The effect of cultivar x feeding period interaction on leaf damage ratings

The interaction (which was highly significant) is shown in Table 5.4.

Table 5.4. An interaction table showing the mean leaf damage ratings in 4 single cross hybrids receiving 10 different infestation levels, after 25 days feeding

	INFESTATION LEVEL (larvae/plant)									
CULTIVAR	4.1	8.7	14.6	19.6	25.3	31.0	35.8	42.7	44.9	51.5
F23 x F09	1.06a[1]	1.23d	1.27g	1.50j	1.87m	2.23q	2.37t	3.43w	3.70a	3.70c
F23 x F07	1.60b	1.87e	1.97h	2.21k	2.67n	3.03r	3.50t	3.86w	3.87a	3.83c
M23 x D50	2.03b	2.33e	2.90i	3.43l	3.56o	4.00s	4.00u	4.00x	4.57b	4.63d
M23 x 56	1.83b	2.23e	3.10i	3.57l	4.03p	4.07s	4.46u	4.53y	4.73b	4.93d
Range	0.97	1.10	1.83	2.07	2.16	1.84	2.09	1.10	1.03	1.23

[1]Means in columns followed by the same letter are not significantly different at the 5% level

L.S.D. 5%	Main effect	Interaction
Cultivar	0.31	0.45
Feeding period	0.31	0.46

The main objective of this experiment was to determine the most efficient and economical number of larvae to apply to each plant. The greatest separation of leaf damage ratings occurred between hybrids at the 19.6 and 25.3 infestation levels. For the 19.6 level, leaf damage ratings ranged from 3.57 to 1.50 (a difference of 2.07). They ranged from 4.00 to 1.87 (a difference of 2.16) for the 25.3 level. Other infestation levels produced smaller ranges. Confirmation of previously determined most efficient infestation levels was obtained. Infestation of approximately 20 larvae/plant was thus used routinely in all resistance investigations, with good separation of plant responses to larval damage.

5.1.2. Leaf damage caused to different maize genotypes after varying feeding periods

(i) Materials and methods

The objective of this experiment was to investigate the timing of leaf damage ratings. The materials and methodology of this experiment were similar to that reported in 4.1.1.1. However, only one larval infestation level (a mean of 19.8 larvae/plant) was utilized. The same 6 cultivars were planted on 22nd October 1981, in a randomized complete block design with split plots and 3 replications. The whole plot treatments were the hybrids and the feeding periods were the sub plots. All plants were infested 33 days post emergence.

Leaf damage was recorded 15, 20, 25 and 30 days after infestation on a single plant basis, on a 1 to 5 scale. The data were averaged for each plot.

(ii) Results and discussion

There was a highly significant ($P<0.01$) difference between leaf damage ratings recorded from each cultivar. The differences between damage ratings recorded at each sampling date were also highly significant, as was the interaction.

Table 5.5. Significance of mean leaf damage ratings in 6 maize cultivars, rated after 4 different periods of larval feeding

SOURCE OF VARIATION	F	F Distribution values	
		5%	1%
Cultivars	6.49**	3.33	5.64
Feeding periods	4.69**	2.87	4.41
Cultivar x feeding periods	18.37**	1.94	2.56

C.V. % Whole Plots = 6.8%
Sub-plots = 11.5%

The effect of cultivars on leaf damage

The leaf damage ratings are shown in Table 5.6:

Table 5.6. Mean leaf damage ratings from 6 maize cultivars, averaged over 4 feeding periods

CULTIVAR					
M06	D57 x M06	D57	56 x 58	58	56
2.11a[1]	2.36a	2.37a	3.10b	3.37bc	3.46c

[1] Means followed by the same letter are not significantly different at the 5% level

L.S.D. (5%) = 0.29

These data are discussed fully under the interaction between cultivars and feeding period.

The effect of feeding period on leaf damage

Table 5.7 shows the leaf damage after each feeding period.

Table 5.7. Mean leaf damage ratings recorded after 4 feeding periods, averaged over 6 cultivars

FEEDING PERIOD (days)			
15	20	25	30
2.03a[1]	2.68b	3.09c	3.38c

[1] Means followed by the same letter are not significantly different at the 5% level

L.S.D. (5%) = 0.40

As the feeding period lengthened, so damage increased.

The effect of cultivar x feeding period interaction on leaf ratings

This interaction is shown in Table 5.8.

Table 5.8. An interaction table showing the mean leaf damage ratings in 6 cultivars, recorded after 4 feeding periods

FEEDING PERIOD	CULTIVAR					
(days)	M06	D57 X M06	D57	56 X 58	58	56
15	1.70a[1]	2.04d	1.81g	2.14k	2.15p	2.36t
20	2.10b	2.25de	2.22h	2.99l	3.37q	3.16u
25	2.33b	2.54e	2.66i	3.32l	3.83r	3.87v
30	2.31b	2.63e	2.78i	3.97m	4.16r	4.44w

[1] Means in columns followed by the same letter are not significantly different at the 5% level

L.S.D. 5%	Main effect	Interaction
Cultivar	0.29	0.38
Feeding periods	0.41	0.42

The interaction was highly significant ($P<0.01$). The increase in damage with time can be best illustrated as the percentage increase:

Table 5.9. Percentage increase in leaf damage ratings in 6 cultivars over a 15 day period (15 day rating to the 30 day rating)

	CULTIVAR				
D57 x M06	M06	D57	56 x 58	56	58
28.9	35.8	53.6	85.5	88.1	93.5

The more resistant group of M06, D57 x M06 and D57 did not show as large an increase in leaf damage over time as did the other more susceptible cultivars.

For maximal expression of leaf damage, it is obviously beneficial to undertake leaf damage ratings as late as possible. Generally, leaf feeding damage was rated after 21 days feeding but prior to tassel emergence, which can be as soon as 50 days post-emergence in some early American or European germplasm. It is obviously beneficial in screening such germplasm to infest as soon as possible once plants have reached a height of about 35cm above ground level. This allows maximum expression of leaf damage before the emerging tassel forces larvae out of the whorl.

5.2 GENERAL PLANT DAMAGE ASSESSMENT

The previous section described the basic methodology of rating leaf damage, which is the main form of damage assessment in the search for resistance. The damage however is not restricted to the leaf tissue. After feeding in the whorl, larvae may feed for up to two weeks in the enclosed tassel. They feed there until the tassel starts emerging and they then move over the outside of the plant and bore into the stem or into the ear. They complete their larval life stage in the stem and then pupate. Although the assessment of resistance is generally based on leaf damage ratings, knowledge was required on general plant resistance and general plant damage. Yield loss, in particular, is of prime importance in commercially exploiting plant resistance to *B. fusca*. The following sections investigate various other aspects of plant damage.

5.2.1 Damage caused to maize inbreds by stalk borer larvae

(i) Materials and methods

The major objective of this experiment was to determine whether resistance factors present in the leaf tissue are sufficient to reduce yield losses. The same eleven maize inbreds used previously in 4.1.1.2 were assessed.

The experiment was planted on 8th November 1983 as a completely randomized block design with split plots and four replications. The whole plot treatments were two infestation levels (0 and 20 larvae/plant), and the sub-plot treatments were the 11 inbreds. Each replicate of an inbred consisted of two rows, with each row containing 10 plants, giving a total of 80 plants for each inbred. Barrier rows were densely planted between treatment rows in order to prevent larval migration into adjacent treatment plants. All plants were infested 29 days post emergence with approximately 20 first instar larvae (mean of 19.7 larvae/plant over the whole trial) applied down the funnel by means of a 'Bazooka'.

Leaf damage was assessed by visually rating the extent of leaf

damage on each plant on a scale of 1 to 5 after 24 days of larval feeding. The ears were harvested and shelled on a plot basis (20 plants), the grain weighed and the moisture content determined. The final yields are expressed as mean yield in g/ plant, adjusted to 12.5% moisture mass which is a standard used by Pioneer Seed Company in yield trials. The heights of infested plants were also compared with the heights of the uninfested plants by visually rating the stunting of the infested plants on a scale of 1 to 5 (1 = very little stunting, 5 = severe stunting). The stems of all the plants were split immediately after harvest, and internal damage to each plant was rated on a scale of 0 to 9 (0 = nil damage, 9 = severe damage).

(ii) Results and discussion

(a) Leaf damage

Table 5.10 summarizes the Analysis of Variance, comparing the mean leaf damage ratings over all inbreds.

Table 5.10. Significance of mean leaf damage ratings of 11 inbreds, recorded after 24 days feeding

SOURCE OF VARIATION	F	F distribution values	
		5%	1%
Inbreds	25.31**	2.18	3.00
C.V.% Plots	= 10.8%		

Highly significant differences ($P<0.01$) were observed between the leaf damage ratings of the different inbreds (Table 5.11).

Data recorded in 4.1.1.2 are included in Table 5.11 for comparison. Although the damage was slightly more severe in the 1983 infestation, there is a similar trend between the two seasons' ratings, and a useful range of damage ratings occurred.

Table 5.11. Mean leaf damage ratings for 11 maize inbreds, after 24 days feeding

	INBRED										
	F23	F03	D57	D50	D55	F08	D54	M23	D53	K11	56
1983	1.84	1.99	2.01	2.38	2.59	2.71	3.26	3.60	3.67	3.69	4.00
	a[1.]	ab	ab	bc	c	c	d	d	d	d	d
1982	1.53	1.48	1.83	1.94	2.34	2.05	2.93	2.63	2.92	3.20	3.71
	p[2.]	p	pq	q	rs	qr	tu	st	tu	u	v
Mean:	1.68	1.73	1.92	2.16	2.47	2.38	3.09	3.11	3.29	3.45	3.85

Means in each row followed by the same letter are not significantly different at the 5% level

[1.] 1983 L.S.D. (5%) = 0.45 C.V. = 10.8%

[2.] 1982 L.S.D. (5%) = 0.38 C.V. = 9.7%

(b) Stunting of infested plants

The effect of stalk borer on plant growth was assessed by rating the difference in height between the infested plants and uninfested plants of the same inbred line (Table 5.13). Highly significant differences were apparent between the inbred lines with regard to stunting.

Table 5.12. Significance of mean height reduction ratings of 11 inbreds recorded at tasselling.

SOURCE OF VARIATION	F	F distribution values	
		5%	1%
Inbreds	16.49**	2.18	3.00

C.V.% Plots = 24.3%

Table 5.13. Mean height reduction ratings of 11 inbreds recorded at tasselling

INBRED										
F08	F03	D57	D55	F23	D54	D50	M23	D53	K11	56
1.25 a[1]	2.25 b	2.25 b	2.50 b	3.00 bc	3.00 bc	3.24 bc	3.51 c	4.75 d	5.00 d	5.00 d

[1] Means followed by the same letter are not significantly different at the 5% level

L.S.D. (5%) = 1.00

Significant differences were apparent between the inbred lines with regard to stunting. F08, F03 and D57 showed very little stunting from stalk borer damage, and D53, K11 and 56 showed severe stunting. With the exception of F08 (which had intermediate leaf damage but very little stunting) there was good correlation between leaf damage ratings and stunting ($r = +0.53$, $P<0.01$) -- if the value for F08 is deleted, $r = +0.74$ ($P<0.01$). During the leaf feeding period (21-35 days) no measurements of height reduction were recorded, but it was evident that little, if any stunting was occurring. Stunting was therefore not causally related to leaf feeding in this experiment, but was due to stem boring activity which occurred

as early as 21 days post infestation in some inbreds, and as late as 35 days in others.

(c) Stem damage

Table 5.14 summarizes the results of the Analysis of Variance comparing the mean stem damage ratings taken at harvest.

Table 5.14. Significance of mean stem damage ratings (taken at harvest) of 11 inbreds after uncontrolled feeding by stalk borer larvae

SOURCE OF VARIATION	F	F distribution values	
		5%	1%
Inbreds	4.79**	2.18	3.00

C.V.% Plots = 23.5%

Table 5.15 shows the ratings for each inbred.

Table 5.15. Mean stem damage ratings at harvest of 11 inbreds infested with stalk borer

INBRED										
F03	D57	F08	D55	D50	M23	D54	F23	56	D53	K11
3.54	3.73	4.49	4.63	5.33	5.88	6.00	6.45	6.74	6.82	8.30
a[1]	ab	abc	abcd	abcde	cde	cde	de	e	e	f

[1] Means followed by the same letter are not significantly different at the 5% level

L.S.D. (5%) = 1.90

There were highly significant differences between the stem damage ratings of the inbreds. The correlation coefficient between stem and leaf damage was significant in all inbreds ($r = +0.54$, $P<0.01$). An exception was the inbred F23, which showed very little leaf damage (1.84 rating), yet showed substantial stem boring (6.45 rating). This phenomenon is possibly due to the stem tissue of F23 having a low nutritional value, resulting in larvae having to consume a large amount of tissue in order to reach the pre-pupal stage. Further research into stem resistance / susceptibility would shed light on the interpretation of differences in stem damage observed in different maize genotypes.

Some of the inbreds that suffered severe stem damage were also severely stunted (56, D53 and K11). The least stunted inbreds (F08, F03, D57 and D55) also showed the least stem damage. The correlation between stunting and stem damage was highly significant ($r = +0.73$, $P<0.01$). As will be described later, the yields of the worst stem damaged inbreds were also significantly the most reduced.

Larvae fed in whorl tissue until either the emergence of the tassel forced them to migrate out of the funnel and into the stem to continue feeding, or until they reached the pre-pupal stage. They then moved out of the whorl into the stem to pupate, irrespective of whether the tassel had emerged. In case larval movement out of the funnels was due to early tassel emergence, the dates of tassel emergence were noted on all inbreds and are shown in Table 5.16 along with the stem damage ratings.

Table 5.16. Age of 11 inbreds at tassel emergence and extent of stem damage caused by stalk borer larvae

INBRED[1.]	PLANT AGE[2.] TO TASSELLING	STEM DAMAGE[3.] RATINGS	DAYS FROM INFESTATION TO 50% TASSELLING
F03	49	3.54a	21
D57	68	3.73ab	37
F08	71	4.49abc	44
D55	63	4.63abcd	35
D50	70	5.33abcde	42
M23	63	5.88cde	35
D54	49	6.00cde	21
F23	49	6.45de	21
56	70	6.74e	42
D53	70	6.82e	42
K11	73	8.30f	45

[1.] Inbreds arranged in order of increasing stem damage
[2.] Days from plant emergence to 50% tassel emergence
[3.] Mean ratings followed by the same letter are not significantly different at the 5% level

It is evident that early tassel emergence, which forced larvae out of the whorl,was not causally related to severe stem damage. The inbreds F03 and F23 both had 50% tassel emergence 21 days after infestation, yet had significantly different stem damage ratings of 3.54 and 6.45. As both these inbreds showed similar leaf damage ratings, the higher stem damage rating of F23 was not due to larger larvae moving out of the whorl of F23. In 4.1.1.2 it was recorded that larvae feeding in F23 were actually smaller than larvae feeding in F03 (Table 4.1.21).The differences in stem damage were obviously due to differences in resistance in the two inbreds. Two other inbreds, F08 and K11, had tassels emerge 44 and 45 days respectively after infestation, yet had

significantly different stem damage ratings of 4.49 and 8.30. It is probable that differences in susceptibility or resistance of the stem tissue were responsible for the widely differing stem damage ratings in those inbred lines which had similar leaf damage ratings, and had stem boring commencing at the same time.

(d) Yield

The effect on yield, of larvae feeding in leaf and stem tissue of the inbreds was investigated by comparing the mean yields of infested and uninfested plants (Table 5.17 and 5.18).

Table 5.17. Significance of percentage yield loss of 11 inbreds after uncontrolled feeding by stalk borer larvae

SOURCE OF VARIATION	F	F distribution values	
		5%	1%
Inbreds	10.56**	2.18	3.00

C.V.% Plots = 19.6%

Table 5.18. Mean grain yields (g/plant) of 11 inbreds, taken from infested and uninfested plots

Inbred[1.]	MEAN YIELD/PLANT (g)		Yield Reduction %
	Uninfested	Infested	
D54	46.69	28.68	38.57a[2.]
D55	38.26	21.50	43.80ab
F08	52.76	24.84	52.92abc
D57	66.55	31.04	53.36abc
F03	34.08	13.51	60.36bc
D50	54.75	17.91	67.29cd
M23	60.62	9.44	84.43de
F23	47.46	6.92	85.42de
D53	40.77	3.14	92.29e
K11	40.46	2.92	92.78e
56	39.91	0.00	100.00e

[1.] Arranged in order of increasing yield reduction.
[2.] Means followed by the same letter are not significantly different at the 5% level

L.S.D. (5%) = 19.70

There were significant differences between the yields of infested and uninfested plants of all inbreds. There were also significantly different amounts of yield loss between inbreds. The lowest yield loss occurred in D54 (38.57% loss) and the highest yield loss occurred in 56 (100.00%). The latter inbred was severely stressed and stunted and produced very few ears, none of which bore any grain. The correlation between yield and leaf damage was significant ($r = +0.39$, $P<0.01$). This was low, as expected, as very little leaf area was lost through larval

feeding, which therefore probably had very little effect on yield. However, once larvae moved into the stem tissue, severe damage resulted in loss of water and nutrient flow, hence the highly significant linear relationship between yield and stem damage ($r = +0.56$, $P<0.01$).

From these results it is evident that although previous research has shown leaf damage ratings to be a quick and efficient field method of selection for resistance to first generation stalk borer, it reflects only the resistance factors present in the leaf whorl. Apart from feeding on leaf tissue, *B. fusca* larvae may also feed for a short time on the enclosed tassel, and also for varying periods in stem tissue. Both sites have been shown to have varying degrees of susceptibility or resistance to the larvae and affect larval development and hence yield loss. In answer to the question as to the efficacy of leaf resistance providing protection against yield loss, it is evident that resistance factors present in the stem tissue and duration of the larval feeding period in stem tissue are additional critical components in a complex interaction of several factors affecting yield.

The splitting of stems is a time-consuming task and impractical under field conditions where thousands of plants have to be evaluated each season in a HPR programme. Yield is an easy component to assess at harvest, and so is stunting of plants if uninfested control plants are available. Because of the good correlation between leaf and stem damage, and between stem damage, stunting and yield, a quick and effective field method of selection for resistance to whorl-feeding *B. fusca* larvae should include a rating of leaf damage after at least 24 days feeding in the whorl, a comparison of stunting between infested and uninfested plants of the same genotype, and an assessment of yield *per se*.. Plants with low leaf damage, little stunting, and good yield are the obvious choices for selection. It is probable that certain genotypes would show high leaf damage coupled with low stem damage and good yield. However, for the presence of

resistance to *B. fusca* in maize hybrids to be appreciated by the farmer growing such a hybrid, it would be of paramount importance for the farmer to see reduced leaf damage. This leaf damage is often the only criterion a farmer will consider as to the effectiveness of resistance to first generation stalk borer feeding in the whorl. It is therefore most important that leaf resistance be present in any commercialised borer-resistant hybrid.

5.2.2. Damage caused to maize single cross hybrids by stalk borer larvae.

This experiment had three objectives :

(i) To determine whether leaf feeding in hybrids has any effect on stunting of plants, and
(ii) To investigate the causes of yield loss in maize hybrids under borer attack, and
(iii) To determine the yield loss of hybrids that were infested.

(i) Materials and methods.

Ten single cross hybrids were made up from previously tested inbreds (see 4.1.1.2.; 5.2.1) and previously tested single cross hybrids (see 4.1.1.3.; 4.2.1.; 4.2.2.). These were chosen to give a good range of damage symptoms. The experiment was planted in a randomized complete block on 30th October 1984, with split plots and 3 replications. The whole plots (20 plants) were two infestation levels (0 and 20 larvae/plant) and the sub-plots were the hybrids. A barrier row of plants was planted between the infested and uninfested rows. The hybrids were infested 35 days later on 2nd December 1984 with a mean of 21.7 larvae/plant.

Leaf damage was assessed after 24 days feeding by rating individual plants on a 1 to 5 scale. Plant height was recorded weekly from 14 to 42 days after infestation. Height was measured from the ground to the end of the leaves extended to their fullest length above the plant.

A stem boring rating was taken at harvest by splitting each stalk and rating the tunneling damage on a 1 - 9 scale. Yield was determined as the mass of shelled grain converted to 12½ % moisture.

(ii) Results and discussion.

(a) Leaf damage

Table 5.19 summarizes the Analysis of Variance, comparing the mean leaf damage ratings over all hybrids.

Table 5.19. Significance of mean leaf damage ratings of 10 single cross hybrids, recorded after 24 days feeding

SOURCE OF VARIATION	F	F distribution values	
		5%	1%
Hybrids	27.42 **	2.30	3.29

C.V. % Plots = 9.6 %

These highly significant differences ($P<0.01$) are presented in Table 5.20.

Table 5.20. Mean leaf damage ratings for 10 single cross hybrids, after 24 days feeding

HYBRID									
F23 x F09	F23 x F03	F03 x M23	F09 x F04	F07 x D54	F23 x F04	F23 x F07	K80 x D50	56 x K80	56 x D50
1.84 a[1]	1.97 a	2.23 ab	2.46 bc	2.53 c	2.56 c	2.70 c	3.29 d	3.82 e	4.67 f

[1] Means followed by the same letter are not significantly different at the 5% level.

L.S.D. (5%) = 0.28

Two of these hybrids (F23 x F09 and F23 x F07) were previously screened together (5.1.1. : Table 5.4). In that experiment, the mean leaf damage ratings for the hybrids after 25 days feeding (mean of 19.6 larvae/plant) were 1.50 and 2.21 respectively. These compare favourably with 1.84 and 2.70 in this experiment, showing good correlation over seasons. The leaf damage, for unknown reasons, was more severe in 1984 than in 1981, and it was evident that a good range in susceptibility occurred.

(b) Plant height

Table 5.21 summarizes the results of the Analysis of Variance, comparing the differences in heights over time between hybrids, and between infested and uninfested plants of the same hybrid.

Table 5.21. Significance of mean heights of infested and uninfested plants of 10 single cross hybrids over time

SOURCE OF VARIATION	F	F distribution values	
		5%	1%
Hybrids	320.85 **	2.05	2.68
Infestation levels	588.33 **	3.95	6.97
Hybrids x infestation levels	28.13 **	2.03	2.74
Time of rating	1047.83 **	3.84	7.01
Hybrids x time	927.98 **	1.68	2.04
Infestation x time	141.94 **	2.48	3.56
Hybrids x time x infestation	8.03 **	1.67	2.04

C.V. % Time = 1.0 %
Time. Hybrids. = 2.9 %
Time. Hybrids. Infestation. = 4.2 %

As expected there were highly significant differences between heights of all hybrids (as all hybrids were genetically different), heights of infested and uninfested plants of the same hybrid (indicating differences in susceptibility), and between the heights recorded at the different times. In addition, all interactions were also highly significantly different. No benefit will be gained by discussing the significance of the difference in mean hybrid heights over all treatments as there are too many interacting factors. Instead, the discussion will investigate (a) the differences in height for each hybrid between its infested and uninfested plants, and (b) the differences in the rate of stunting over time for all hybrids.

Height differences between hybrids (infested vs uninfested plants).

Table 5.22. Final heights (at anthesis) of infested and uninfested plants of 10 single cross hybrids

HYBRID	FINAL PLANT HEIGHT (CM)		% HEIGHT DIFFERENCE	
	UNINFESTED	INFESTED		
F23 x F09	197.3	180.0	8.8	*
F03 x M23	180.0	163.4	9.3	*
F09 x F04	203.8	173.6	14.8	*
F07 x D54	191.5	162.2	15.3	*
F23 x F03	189.8	160.3	15.5	*
F23 x F04	163.9	137.4	16.2	*
F23 x F07	214.6	169.5	21.0	*
56 x D50	183.4	133.9	23.9	*
56 x K80	294.6	204.8	30.0	*
K80 x D50	302.1	192.8	35.7	*
MEAN	211.0	167.8	20.5	*

L.S.D. 5 %	Main effect	Interaction
Hybrids	7.94	14.99
Infestation	4.73	13.20

There is no point in discussing the significant differences between hybrids (for the uninfested or infested values) as they are all genetically different, and the heights are not comparable. What is important are the differences between uninfested and infested plants of the same hybrids, shown in Table 5.22 as the percentage height loss.

The hybrid least affected was F23 x F09 (8.8 % height reduction) and the most susceptible hybrid was K80 x D50 (35.7 %) In 4.2.1. and 4.2.2. out of 3 hybrids, the hybrid K80 x D50 was also the most susceptible. It had the highest number of larvae/plant, the heaviest larvae, and the greatest larval biomass/plant. Its general susceptiblity is again demonstrated in this experiment. As will be discussed, the length of the growing period and the amount of stem damage have strong influences on the extent of stunting.

Differences in the rate of stunting over time

(i) 14 days feeding.

Table 5.23. Mean heights of infested and uninfested plants of 10 single cross hybrids after 14 days feeding

	PLANT HEIGHT (cm)		
HYBRID	Uninfested	Infested	
F23 x F04	89.80	91.77	N.S.
56 x D50	102.63	101.50	N.S.
F23 x F07	116.33	118.93	N.S.
F09 x F04	117.53	116.90	N.S.
F07 x D54	118.60	113.27	N.S.
F23 x F03	122.13	120.90	N.S.
56 x K80	122.17	119.33	N.S.
F03 x M23	123.00	120.10	N.S.
F23 x F09	123.13	123.70	N.S.
K80 x D50	131.00	132.00	N.S.
MEAN	116.63	115.84	N.S.

L.S.D. 5 %	Main effect	Interaction
Hybrids	6.69	9.36
Infestation	2.91	9.34

C.V. % Hybrids = 3.4 %
Hybrids. Infestation = 4.7 %

There were very small insignificant differences between the heights of infested and uninfested plants of each hybrid after 14 days leaf feeding. Some hybrids, probably because of uneven ground and slightly variable growth patterns of each plant,

showed reduced height in the uninfested plants compared with the infested plants. Obviously larvae feeding in the whorl for up to 14 days had no adverse effect on the growth of the plant.

21 days feeding

As with the data recorded after 14 days feeding, minor stunting occurred in the hybrids after 21 days leaf feeding. Larvae were feeding extensively in leaf tissue and evidently leaf damage of the severe nature noted in 56 x 80 and 56 x D50 (see Table 5.20) was having no effect on plant growth.

Table 5.24. Mean heights of infested and uninfested plants of 10 single cross hybrids after 21 days feeding

	PLANT HEIGHT (cm)		
HYBRID	Uninfested	Infested	
F23 x F04	116.47	121.53	N.S.
56 x D50	125.00	123.03	N.S.
F03 x M23	142.43	146.20	N.S.
F07 x D54	147.10	141.90	N.S.
F23 x F07	147.37	148.93	N.S.
F23 x F03	152.90	150.53	N.S.
F09 x F04	154.67	152.23	N.S.
F23 x F09	159.40	156.00	N.S.
56 x K80	161.67	163.73	N.S.
K80 x D50	173.97	167.13	N.S.
MEAN	148.11	133.42	N.S.

L.S.D. 5 %	Main effect	Interaction
Hybrids	8.08	6.88
Infestation	2.17	9.38

C.V. % Hybrids = 3.2 %
Hybrids. Infestation = 2.7 %

28 days after infestation

By 28 days after infestation some hybrids started showing significant differences in height between infested and uninfested plants.

Table 5.25. Mean heights of infested and uninfested plants of 10 single cross hybrids 28 days after infestation

	PLANT HEIGHT (cm)		
HYBRID	Uninfested	Infested	
F23 x F04	140.27	136.73	N.S.
56 x D50	147.63	130.53	*
F03 x M23	164.83	158.33	N.S.
F07 x D54	171.77	158.77	*
F23 x F07	175.30	170.40	N.S.
F23 x F03	180.13	157.93	*
F09 x F04	184.27	171.27	*
F23 x F09	185.83	182.23	N.S.
K80 x D50	202.40	182.73	*
56 x K80	203.03	187.77	*
MEAN	175.55	163.67	*

L.S.D. 5 %	Main effect	Interaction
Hybrids	9.21	9.25
Infestation	2.93	11.25

C.V. % Hybrids = 3.2 %
Hybrids. Infestation = 3.2 %

It was during this period (the 4th week after infestation) that tassels started emerging and elongation of most hybrids slowed down. The later maturing hybrids still had a substantial amount of elongation to achieve (up to 46% for K80xD50 -see Table 5.26).

Table 5.26. Weekly percentage increase in height of infested and uninfested plants of single cross hybrids

HYBRID	TREATMENT	DAYS AFTER INFESTATION			
		14 - 21 days	21 - 28 days	28 - 35 days	35 - 42 days
F23 x F09	infested	34.2	9.7	- 0.2	- 0.9
	uninfested	29.4	16.6	5.2	0.9
F23 x F04	infested	32.5	12.5	0.0	0.8
	uninfested	29.7	20.4	14.7	1.8
F09 x F04	infested	30.2	12.5	1.9	- 0.5
	uninfested	31.4	19.1	9.8	0.7
F23 x F07	infested	25.2	14.4	0.7	- 1.2
	uninfested	26.7	18.9	21.1	1.1
F07 x D54	infested	25.2	11.8	2.5	0.0
	uninfested	24.0	16.5	11.1	0.6
F23 x F03	infested	24.5	4.9	1.8	0.0
	uninfested	25.2	17.8	5.2	0.0
F03 x M23	infested	21.7	8.3	2.9	0.3
	uninfested	15.8	15.7	8.7	0.5
56 X K80	infested	37.2	14.6	7.1	1.9
	uninfested	32.6	24.9	41.5	2.4
56 X D50	infested	21.2	6.1	2.6	0.0
	uninfested	21.8	18.1	14.8	3.9
K80 X D50	infested	26.6	9.3	4.6	0.9
	uninfested	32.7	16.4	46.2	1.3

It was also at this time that stem boring started in several hybrids :

Table 5.27. Leaf damage rating, and time of stem boring activity and height reduction after 28 days feeding in 10 single cross hybrids

HYBRID	LEAF DAMAGE RATING	STEM BORING COMMENCEMENT (DAYS AFTER INFESTATION)	% HEIGHT DIFFERENCE	
F23 x F09 [$]	1.84 a[1]	28	1.9	N.S.
F23 x F04 [$]	2.56 c	28	2.6	N.S.
F23 x F07 [$]	2.70 c	26	2.8	N.S.
F03 x M23 [$]	2.23 b	26	3.1	N.S.
56 x K80	3.82 e	23	7.0	*
F07 x D54 [$]	2.53 c	23	7.4	*
F09 x F04 [$]	2.46 bc	22	7.5	*
K80 x D50	3.29 d	21	9.7	*
56 x D50	4.67 f	21	11.6	*
F23 x F03 [$]	1.97 a	21	12.3	*

[1] Means followed by the same letter are not significantly different at the 5% level
[$] Tassel emergence ocurred during the period 21 - 28 days after infestation.

It is clear from this table that the significant reduction in height recorded in some hybrids was due to stem boring and not to leaf feeding. The earlier the stem boring, the more severe the stunting. Up to the time of commencement of stem boring, leaf feeding did not cause any stunting.

Tassel emergence will always cause larvae feeding in whorl tissue to migrate out of the whorl and bore into the stem. Depending on how much more development the larvae have to undergo in order to pupate, stem boring activity may range from negligible to

extensive. The closer larvae are to pupating, the less feeding (hence damage) occurs.

The larvae feeding in these hybrids after 28 days were obviously still affecting growth, as evidenced by the height reduction in the last few hybrids in Table 5.27. Larvae had however migrated of their own accord in some hybrids, as evidenced by significant height loss in some hybrids that had not yet reached the tasselling stage (56 x D50, K80 x D50, 56 x K80). It is interesting to note that the 3 susceptible inbreds (56, D50 and K80) occur in these hybrids in all combinations. Development of larvae was obviously rapid in these susceptible hybrids and larvae had migrated out of the plant whorl to pupate and had bored into the stem, causing a disruption to stem elongation. The picture is clear with those hybrids which had tasseled. Some of them (F23 x F03, F09 x F04, and F07 x D54) showed significant height loss as expected, as larvae would have had to continue their development in stem tissue, thus causing loss in water and nutrient flow. The stunting in 56 x D50 and K80 x D50 occurred as these hybrids were still elongating (see Table 5.26).

The other four hybrids (F23 x F09, F23 x F04, F23 x F07, F03 x M23) had just begun to show stem boring after 26-28 days after infestation, and therefore showed insignificant height reduction.

Without having removed larvae from the stem tissue, it is nontheless of interest to comment on the causes of the stunting. As was seen with many previous experiments, low leaf damage was generally caused by few or small larvae feeding in the leaf tissue (low larval biomass). Low leaf damage in F23 x F09 (a rating of 1.84) obviously resulted from a low larval biomass. The small amount of stunting could have resulted from this small biomass, but more likely from the late and minor amount of stem boring (on the day of height measurement). One could explain the insignificant height reduction in the next 3 hybrids (F23 x F04, F23 x F07, F03 x M23) in a similar way. However, F23 x F03 had

very little leaf damage, yet had significant stem damage and height reduction. This was probably due to stem boring occuring very early, coupled with larvae feeding in susceptible stem tissue.

As will be discussed later, major factors in yield loss are : the time that stem boring occurred relative to the stage of maturity of the hybrid, the size of larvae, and the level of susceptibility of the stem tissue.

35 days after infestation

By this stage extensive stem boring had occurred. Hybrids were still elongating, but at a slower rate than previously (see Table 5.26).

Table 5.28. Mean heights of infested and uninfested plants of 10 single cross hybrids 35 days after infestation

Hybrid	Plant height (cm)		
	Uninfested	Infested	
F23 x F04	161.0	136.2	*
56 x D50 $	169.5	133.9	*
F03 x M23	179.1	162.9	*
F23 x F03	189.5	160.7	*
F07 x D54	190.4	162.6	*
F23 x F09	195.5	181.8	N.S.
F09 x F04	202.3	174.5	*
F23 x F07	212.3	171.6	*
56 x K80 $	286.0	201.0	*
K80 x D50 $	295.9	191.1	*
MEAN	208.1	167.6	*

$ Tassel emergence occurred during the period 28-35 days after infestation.

L.S.D. 5 %	Main effect	Interaction
Hybrids	8.22	15.52
Infestation	4.90	13.68

C.V. % Hybrids = 2.6 %
Hybrids. Infestation = 4.8 %

There were large differences in some hybrids between the infested and uninfested plants, as it was during this period (28-35 days) that the greatest amount of plant elongation took place (see

Table 5.26). It was also the time when most stem boring, and hence interruption of nutrient and water flow, occurred. Obviously those late maturing hybrids which still had a certain amount of growth to attain were the most affected by having nutrient flow disruption. Only F23 x F09 did not show a significant height loss, indicating possibly resistant stem tissue.

42 days after infestation

Increases in plant height were not significant by this stage (Table 5.26) as all tassels had emerged during the previous two weeks. There was also no increase in heights of hybrids after 42 days and up to post pollination stage of growth.

Table 5.29. Mean heights of infested and uninfested plants of 11 single cross hybrids 42 days after infestation

Hybrid	Plant height (cm) Uninfested	Infested	
F23 x F04	163.9	137.4	*
56 x D50	176.1	133.9	*
F03 x M23	180.0	163.4	*
F23 x F03	189.8	160.3	*
F07 x D54	191.5	162.2	*
F23 x F09	197.3	180.0	*
F09 x F04	203.8	173.6	*
F23 x F07	214.6	169.5	*
56 x K80	292.8	204.8	*
K80 x D50	299.8	192.8	*
MEAN	211.0	167.8	*

L.S.D. 5 %	Main effect	Interaction
Hybrids	7.94	14.99
Infestation	4.73	13.20

C.V. % Hybrids = 2.4 %
Hybrids. Infestation = 4.6 %

The rate of stunting was significant, but not as marked as previously due to the slow down of plant growth (Table 5.26). The reduction in height caused to each hybrid by larvae feeding in stem tissue is clearly illustrated in Fig. 18.

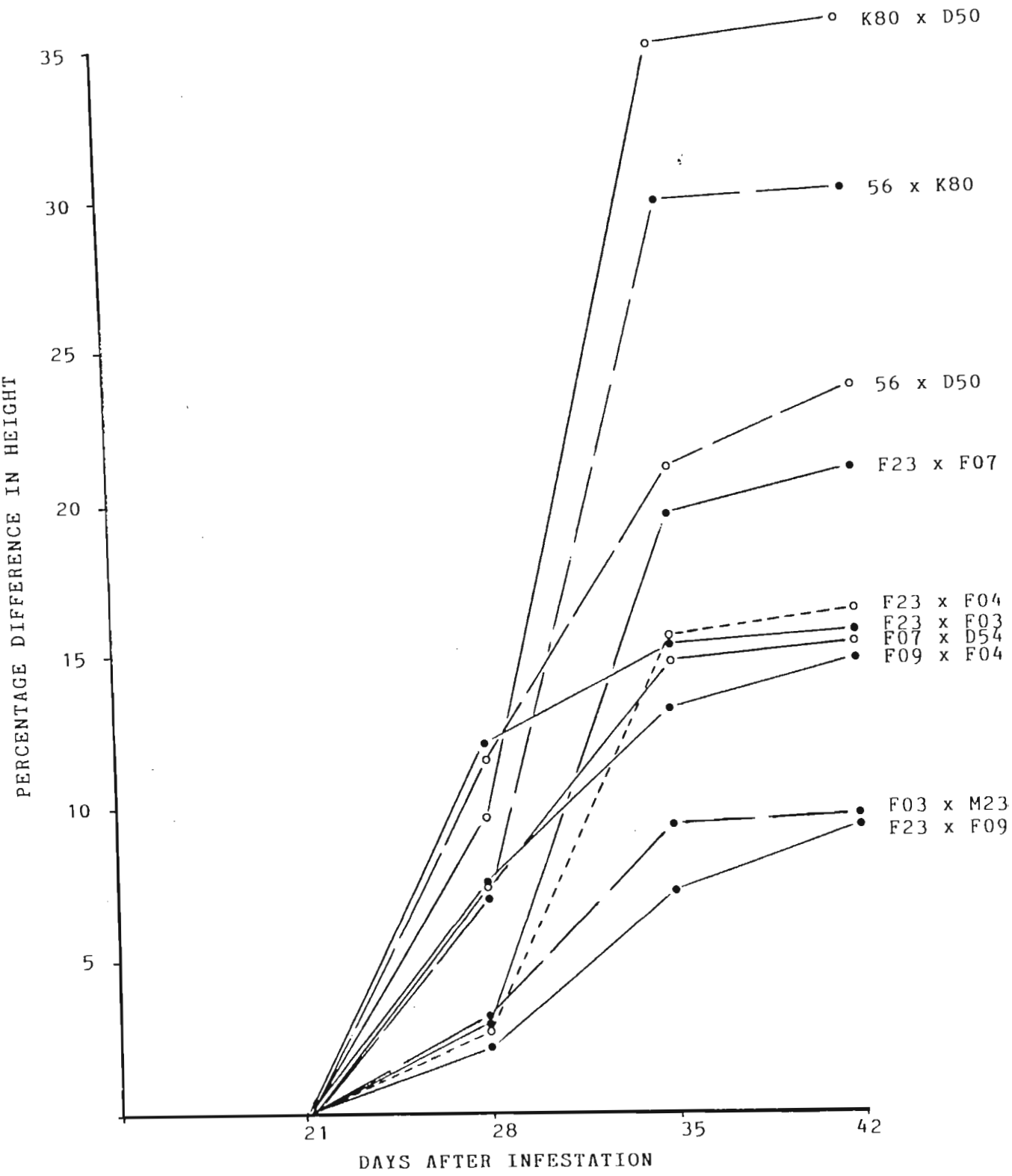


Fig. 18. Height reduction in 10 single cross hybrids, expressed as the % difference in height between the infested plants and the uninfested plants for each hybrid.

It is evident that interpretation of height reduction in hybrids infested by stalk borer is no simple matter. Factors that have a great bearing on height loss are :

(i) The stage of larval development at the time of stem boring. If the larvae were small and still had a considerable amount of feeding to go through to attain the pre-pupal stage, then extensive feeding and damage would occur. The influence of leaf resistance would be the primary determining factor in the rate of initial development of larvae.

(ii) the larval biomass entering the stem. Obviously the larger the biomass, the more immediate damage the larvae can cause. However, if a group of small larvae (growth suppressed due to resistant leaf tissue) enter the stem, and the stem tissue is susceptible, then extensive stem feeding may take place. This would result in even greater damage than that caused by the larger larval biomass. So larval biomass is only one of many factors affecting stunting.

(iii) The stage of plant development at the time of stem boring. The younger the plant, the more it has to grow, and the more its growth would be stunted.

(iv) The level of resistance in the stem tissue to the larvae would also determine the growth of the larvae and hence have an effect on the extent of stem damage.

(v) The maturity of the hybrid (short, medium or long season). Early infestations in short season hybrids will have a smaller effect than in a late maturing hybrid.

(vi) Finally, the physiology of the plant would have an effect on how much it was affected by the damage. Presumably thicker stemmed plants could sustain higher levels of damage than thin stemmed plants. This could certainly be considered as tolerance, and would be a most useful attribute in any hybrid.

The whole picture is further complicated by the effect of the damage on grain yield, which is discussed later.

(c) Stem damage

The Analysis of Variance, comparing the mean stem damage ratings over all hybrids is summarized in Table 5.30.

Table 5.30. Significance of mean stem damage ratings of 10 single cross hybrids, recorded at harvest

SOURCE OF VARIATION	F	F distribution values	
		5 %	1 %
Hybrids	24.55 **	2.30	3.29
C.V. % Plots	= 8.5 %		

These highly significant differences ($P < 0.01$) are presented in Table 5.31.

Table 5.31. Mean stem damage ratings taken at harvest for 10 single cross hybrids

HYBRID									
F03 x M23	F09 x F04	F23 x F07	F07 x D54	F23 x F09	K80 x D50	56 x K80	F23 x F04	F23 x F03	56 x D50
3.74 a[1]	4.01 a	4.79 ab	5.09 b	5.62 b	5.71 b	7.36 c	7.77 c	7.75 c	9.00 d

[1] Means followed by the same letter are not significantly different at the 5% level

L.S.D. (5 %) = 1.07

A mean rating of 9.00 is a most severe rating, and the bulk of the stem tissue of 56 x D50 was completely destroyed by larvae. The major factors that could have an effect on the amount of stem damage are the size of larvae initially entering the plant stems, the period of feeding before pupation, and the level of resistance inherent in the stem tissue. None of these variables could be measured in this experiment of which a prime objective was yield loss assessment. One can only comment on the actual amount of stem damage without surmising how the differences occurred. What is of great importance is the effect that stem damage has on yield loss, which is discussed next.

(d) Yield

Table 5.32 summarizes the results of the Analysis of Variance, comparing the mean yields of infested and uninfested plants of 10 single cross hybrids.

Table 5.32. Significance of mean yields of infested and uninfested plants of 10 single cross hybrids

SOURCE OF VARIATION	F	F distribution values	
		5%	1%
Hybrids	1375.44 **	2.05	2.68
Infection levels	642.49 **	3.95	6.97
Hybrids x infestation levels	39.95 **	2.03	2.74

C.V. % Hybrids = 8.5 %
Hybrids. Infestation = 12.6 %

These highly significant differences are shown in Table 5.33.

Table 5.33. Mean yield/plant of infested and uninfested rows of 10 single cross hybrids

HYBRID	MEAN YIELD/PLANT (g) Uninfested	Infested	% YIELD LOSS	
F03 x M23	1451.3	1104.6	23.9	*
F23 x F09	941.6	573.6	39.1	*
K80 x D50	2068.0	1238.0	40.1	*
F09 x F04	1775.6	1037.6	41.6	*
F23 x F03	932.7	536.7	42.5	*
F23 x F07	1771.3	894.7	49.5	*
56 x K80	1848.0	869.6	52.9	*
F07 x D54	1150.0	516.7	55.1	*
F23 x F04	762.7	333.0	56.3	*
56 x D50	2029.7	300.3	85.2	*
MEAN	1473.1	740.5	47.3	*

L.S.D. 5 %	Main effect	Interaction
Hybrids	246.5	277.5
Infestation	185.4	341.3

The percentage yield loss varied from 23.9 % for F03 x M23 to as high as 85.2 % for 56 x D50. All hybrids showed a significant yield loss.

It is evident from the previous discussions that the amount of stem boring was the main determining factor in height reduction. Obviously retardation of growth, and presumably reduction of nutrient flow into the developing ears would have a significant

effect on final yields of these hybrids. The analysis of why some hybrids showed a greater yield loss than others is complex. It is reliant on assessment of leaf damage ratings, time of stem boring activity, days to tassel emergence, height of plant, percentage height reduction, stem damage, and yield potential of the plant.

Table 5.34. Summary of data pertinent to yield loss in 10 single cross hybrids

HYBRID	% YIELD LOSS	UNINFESTED YIELD/PLANT(g)	STEM DAMAGE RATING	% HEIGHT REDUCTION	FINAL PLANT HEIGHT(cm)	DAYS TO TASSEL EM [1]	DAYS TO STEM BORING [2]	LEAF DAMAGE RATING
F03xM23	23.9	1451.3	3.74	9.3	180.0	71	26	2.23
F23xF09	39.1	941.6	5.62	8.8	197.3	66	28	1.84
K80xD50	40.1	2068.0	5.71	35.7	302.1	81	21	3.29
F09xF04	41.6	1775.6	4.01	14.8	203.8	71	22	2.46
F23xF03	42.5	932.7	7.75	15.5	189.8	64	21	1.97
F23xF07	49.5	1771.3	4.79	21.0	214.6	71	26	2.70
56 xK80	52.9	1848.0	7.36	30.0	294.6	81	23	3.82
F07xD54	55.1	1150.0	5.09	15.3	191.5	71	23	2.53
F23xF04	56.3	762.7	7.77	16.2	163.9	71	28	2.56
56 xD50	85.2	2029.7	9.00	23.9	183.4	88	21	4.67

[1] Days to tassel emergence from planting.
[2] From infestation.

The analysis of correlation showed the following relationships between all attributes:

Table 5.35. Correlation matrix of various attributes of 10 single cross hybrids

	1	2	3	4	5
1.% Yield loss	1				
2.Stem damage ratings	+0.63 **	1			
3.% Height reduction	+0.52 *	+0.48 *	1		
4.Leaf damage ratings	+0.49 *	+0.50 *	+0.52 *	1	
5.Days to stem boring	+0.14 N.S.	-0.37 N.S.	-0.46 *	-0.48 *	1

5 % r = 0.44
1 % r = 0.56

There were significant correlations between all attributes except days to stem boring and yield loss, and days to stem boring and level of stem damage. These attributes are discussed below. The strongest correlation was between stem damage and yield loss, indicating that assessment of this type of damage is most important in yield loss analysis. However there are some hybrids that do not have strong correlations between the various data recorded. Using yield loss as the most important attribute, it is interesting to analyse these discrepancies.

1. F03 x M23 had the lowest yield loss, due to very little stem damage and stunting. Stem boring started late on this medium maturing hybrid (71 days to tassel emergence) and low leaf damage was also recorded. This is a clearly understood pattern of events, indicating a resistant leaf reaction and possibly resistant stem tissue.

2. F23 x F09, a medium maturity hybrid, had fairly severe stem damage but very little height reduction, due to larvae boring in late. The yield loss should have been higher for the stem damage which occurred, indicating tolerance perhaps, and the ability of this hybrid to still fill out the ears despite fairly severe stem damage.

3. K80 x D50, a late maturing hybrid, with a susceptible leaf reaction, had stem boring occur very early. Height reduction was severe (the greatest loss of all hybrids) and stem damage was also fairly severe. Despite all these susceptible reactions, yield loss was unexplainably intermediate. Possibly the fact that this hybrid was the tallest and most vigorous of all hybrids could indicate tolerance to the type of damage that would normally severely affect a smaller hybrid.

4. F09 x F04, an intermediate maturity hybrid showed intermediate leaf damage, not much height loss and a low stem damage rating. The 41.6% yield loss recorded was therefore as expected.

5. F23 x F03, an early hybrid, showed a resistant leaf reaction, not much height loss, but severe stem damage. The reasonably low yield loss (42.5%) does not correlate well with the high amount of stem damage. As the hybrid is an early cultivar, it is possible that the severe stem damage could have developed after the plant had started its ear development and that stem boring would not have had much effect on yield.

6. F23 x F07, a medium hybrid, showed intermediate leaf damage, stunting and stem damage. The yield loss of 49.5% could be attributed to the levels of stem damage sustained.

7. 56 x K80, a late hybrid, showed a very susceptible leaf reaction, substantial height reduction and severe stem damage. The yield loss of 52.9% correlates well with the damage.

8. F07 x D54, an intermediate maturity hybrid showed an intermediate leaf reaction, fairly severe stem damage but not much stunting. A severe yield loss of 55.1% would therefore indicate that the severe stem damage occurred

after stem elongation was complete, and had the greatest effect on nutrient flow and grain filling.

9. F23 x F04, an intermediate maturing hybrid, had an intermediate leaf reaction, late stem boring, and consequently very little height loss (expected also as this hybrid is a short hybrid). Stem damage was severe (occurring after stem elongation ceased) which caused the high yield loss (56.3%).

10. 56 x D50, a very late maturing hybrid, showed the most susceptible leaf reaction, and had very early stem boring. Height reduction was therefore pronounced, and the severe stem damage caused a great loss of yield. A classic case of a highly susceptible hybrid.

In conclusion, it is evident that damage caused by larvae feeding in the leaf tissue does not contribute to height loss in maize cultivars. Once larvae migrate to the stem and commence stem boring, the damage occurs. The earlier on in the stage of hybrid development that stem boring occurs, the greater the interference with the plant's vital processes, and the greater the stunting and yield loss. One would expect therefore to have more success in developing resistance to *B. fusca* in early material. This is in fact the case, where a great many of the resistant inbreds developed in the breeding programme are early lines. This is discussed more fully in Chapter 6. Van Rensburg *et al.* (1988 f) stated that the longer season cultivars suffered more yield loss than short season cultivars. They also stated that variances in stalk thickness among cultivars were recorded, and said that this and other physical characteristics may play a role in yield loss.

6. DISCUSSION ON PLANT DAMAGE AND INTERACTION BETWEEN LARVAE AND PLANTS

A much clearer picture is now available as to the interaction between *B. fusca* larvae and maize. Because the larvae feed on several plant parts, there are several sites where resistance can play an important role in reducing plant damage. Although first generation larvae sometimes feed on the tassel and occasionally on the ear, feeding occurs predominantly in the whorl and stem. It is while feeding on these last two sites that the most interaction between plant and insect occurs.

Larvae commence feeding in the whorl of the maize plant. It is here that three resistance mechanisms have been identified. One mechanism retards larval growth, resulting in delayed development and hence smaller larvae than in susceptible plants. Another mechanism kills some of the larvae, resulting in a reduction in numbers of larvae. The third mechanism repels larvae, also resulting in a reduction in numbers of larvae. The resultant low larval biomass causes minimal leaf damage.

Larvae feeding in susceptible plants encounter no such resistance, and feed unhindered to maturity. Many large larvae feeding in such a plant cause extensive leaf damage.

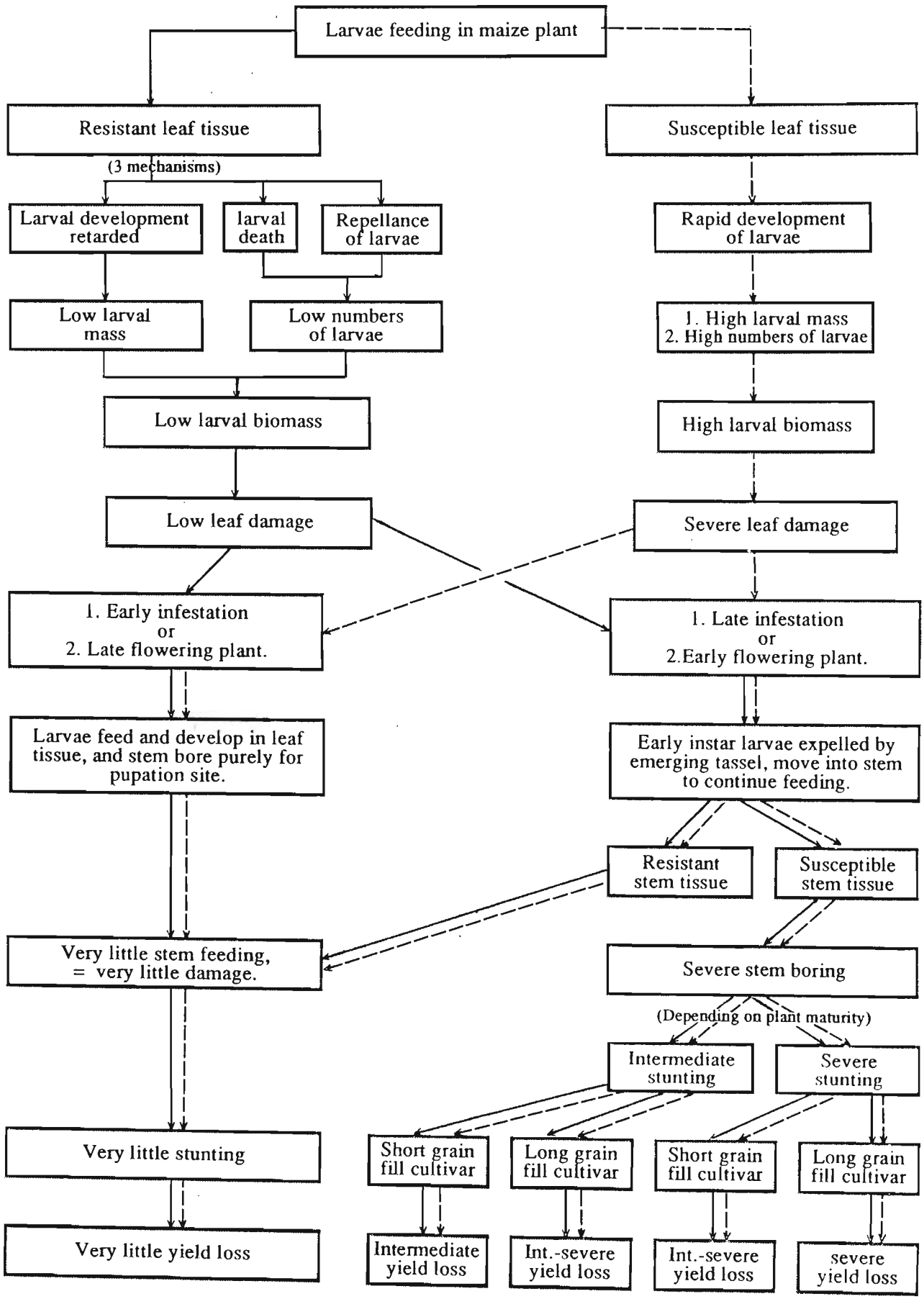
The interaction in the plant whorl between insect and plant is however complicated by several other factors. If the infestation commences when the plants are young, larvae will complete their growth in the whorl tissue, and reach the pre-pupal stage before the tassel emerges. These pre-pupal larvae will then leave the whorl of their own accord, and bore into the stem purely to pupate. A small cavity is bored out, and not much impedance of nutrient flow occurs. Very little stunting results, and so the yield is minimally affected. This above scenario will also occur if the plant under attack is a long season or late flowering cultivar. The larvae will not be forced out of the whorl as they will complete their development prior to tasselling.

The other scenario is one where the infestation occurs late in the plant's stage of development (just prior to flowering), or in an early flowering (short season) hybrid. Larvae manage to feed only for a few days in leaf tissue before being forced out of the whorl and into the stem. These early instar larvae must now complete their development in the stem tissue. It is at this stage that resistant stem tissue will exert an effect. If resistant stem tissue is encountered, very little damage occurs, and the plant continues growing unhindered. Very little stunting occurs so very little yield loss is caused. However if susceptible stem tissue is encountered, extensive feeding takes place. A further complicating factor now enters the interaction. Cultivars differ in the time taken to attain grain maturity. This period, referred to as the grain filling period, can vary substantially (up to several weeks' difference between cultivars). The stem boring caused by larvae will impede the nutrient and water flow to the ear, thus affecting its development. If the cultivar under attack only has a short grain filling period, it will suffer less yield loss than a long grain filling cultivar.

It is obvious that a wide range of plant damage can occur in both resistant and susceptible plants. The anomalous result is that cultivars that show a highly resistant leaf reaction to *B. fusca* may actually end up with a severe loss in yield, and highly susceptible cultivars (leaf reaction) may show very little yield loss.

For the successful commercialization of a resistant hybrid, it is imperative that the farmer sees very little leaf damage. Obviously it is also necessary to develop good stem resistance to counter all eventualities of late or early infestations. It is also obvious that resistance would be easier to obtain in early maturing hybrids, which appears to be borne out in practical experience of developing resistant inbred lines (see Chapter 7).
The interaction is summarized in Fig.19.

Fig. 19. Flow chart illustrating the insect / plant interaction with *B. fusca* larvae feeding in maize.



7. DEVELOPMENT OF RESISTANT GERMPLASM

The prime objective of any HPR programme is to develop insect resistant cultivars for use by commercial farmers. The methodologies involved in mass rearing, infestation and damage assessment have been discussed. Assessment of both plant and insect responses indicated that resistant mechanisms were present in certain maize genotypes. However, to be of use in any maize breeding programme, the resistance must be developed in agronomically sound germplasm for eventual incorporation into commercial hybrids. It is of no use whatsoever to develop resistance to any insect if that resistance cannot be presented in a usable form, usually in inbreds. In addition, populations must also be developed as new sources of improved resistance, from which new inbreds can be developed.

7.1 DEVELOPMENT OF RESISTANT INBREDS

The development of resistant inbred parents involves standard maize breeding techniques, well documented in several books (Jugenheimer, 1976; Sprague, 1977).

The initial germplasm may come from widely varying regions of the world, and have greatly differing characteristics. Because maize is also grown at various altitudes and latitudes, the genetic diversity from which to develop inbreds is vast. The researcher thus has access to a wide range of germplasm. The choice of source populations will therefore have a strong influence on the level of resistance as well as other desirable and undesirable traits in the development of usable inbreds.

The chances of obtaining inbreds that are resistant to a certain pest depend on the frequency of genes conditioning that resistance. The lower the gene frequency, the lower the probability that inbreds extracted from the source population will show any level of resistance. The breeding system used to develop stalk borer-resistant germplasm is the same as that used in the conventional maize breeding programme at Pioneer Seed

Company. The only difference is that the plants are artificially infested with *B.fusca* larvae, and prime emphasis is placed on resistant responses. However, bearing in mind that the eventual goal is the incorporation of resistant inbreds into commercial hybrids, an attempt is made to also select material that is agronomically acceptable.

Commercial hybrids are made by crossing dissimilar homozygous inbreds in various combinations that result in high yielding F1 progeny. Homozygous inbreds have therefore to be developed from heterozygous sources. In the process of inbred development, a method called self pollination is used. This entails covering the emerging silk with a paper bag before any cross pollination can occur. Once the plant starts shedding pollen, the pollen is collected in a packet, the silk cover is removed, and the plant has its own pollen placed on its own silk. The process is termed selfing, or self pollinating. With the first self, the resultant seed is 50% homozygous, and with each successive selfing, the percentage homozygosity increases : selfed twice (75%), selfed three times (87.5%), selfed four times (93.7%), selfed five times (96,8%). After 4 selfs, the homozygosity is considered stable enough to utilize in hybrid combinations.

The development of resistant inbreds from source populations follows a straightforward path. A sample of, say, 1000 plants from each of several heterozygous populations will be planted out in Year 1. The plants will be infested, rated in a field book and self pollinated. At harvest, selection of the best 20-30% occurs, taking into account the resistance rating and other agronomic attributes. The selfed seed (F2 or S1) is planted out (ear to row) in Year 2. The plants are infested, rated, self pollinated and the best 10-20% selected. The cycle is repeated for a 3rd year, again selecting the best 10-20% of the plants. In the 4th year, the S3 selections ("initial selections") are crossed onto two elite tester inbreds. The resultant single cross hybrids are then yielded in Year 5 in replicated trials at 2 or 3 localities. The yield trial results cover not just yield, but standability,

cob disease, shelling percentage, moisture, leaf diseases and colour. Selection of the best 10% of these initial S3's is made, based on all the above criteria as well as data on resistance. These selected inbreds are then termed "intermediate" inbreds, and the same S3 selections are again crossed (Year 6) to testers, but this time to 4 testers, to give a better indication of their combining ability. The remnant seeds of the S3 inbreds are also planted and self pollinated to the S4 stage.

The single crosses between the intermediates and the tester parents are planted out in Year 6 at 9 sites to expose them to even more varied environments. In Year 7, the yield results are obtained, and the best 10% of the intermediates are selected, and termed "advanced" inbreds. These advanced inbreds are crossed in Year 8 with 8-10 elite tester parents, and the single crosses yielded at 9 sites in Year 9.

By this stage, the advanced inbreds have been thoroughly tested against a wide variety of elite tester parents and at several localities. Confirmation of the level of resistance has also been obtained. A good idea as to their usefulness is now available. From the single cross data available over several seasons, hybrids are made up by the maize breeders utilizing the advanced inbreds in the best combinations. This takes place in Year 10, and often in Year 11 if the correct single cross hybrids are not available and have to be remade.

The hybrids are then tested extensively throughout South Africa over a 3 year period before being commercialised. The path from the initial utilization of germplasm to eventual use of inbreds in commercial hybrids is thus a long one.

The initial sources of germplasm utilized in any HPR programme should be the best adapted material possible, normally elite ("advanced") inbreds. These inbreds have been developed by maize breeders for use in commercial hybrids, and are the culmination of many years of intensive plant breeding. If any resistance can

be identified in elite germplasm, the goal of developing agronomically acceptable resistant hybrids is readily attainable. If elite material shows no resistance, then the search continues onto "intermediate" and untested inbreds. If none of those inbreds show any usable resistance, then the search must move onto elite populations, from which resistant inbreds can be developed after several years of breeding. Failing the discovery of any usable resistance in elite populations, exotic unadapted germplasm must now be investigated. The further down the line one investigates, the longer it will take to develop elite, resistant, agronomically acceptable material that can be used to make resistant commercial hybrids.

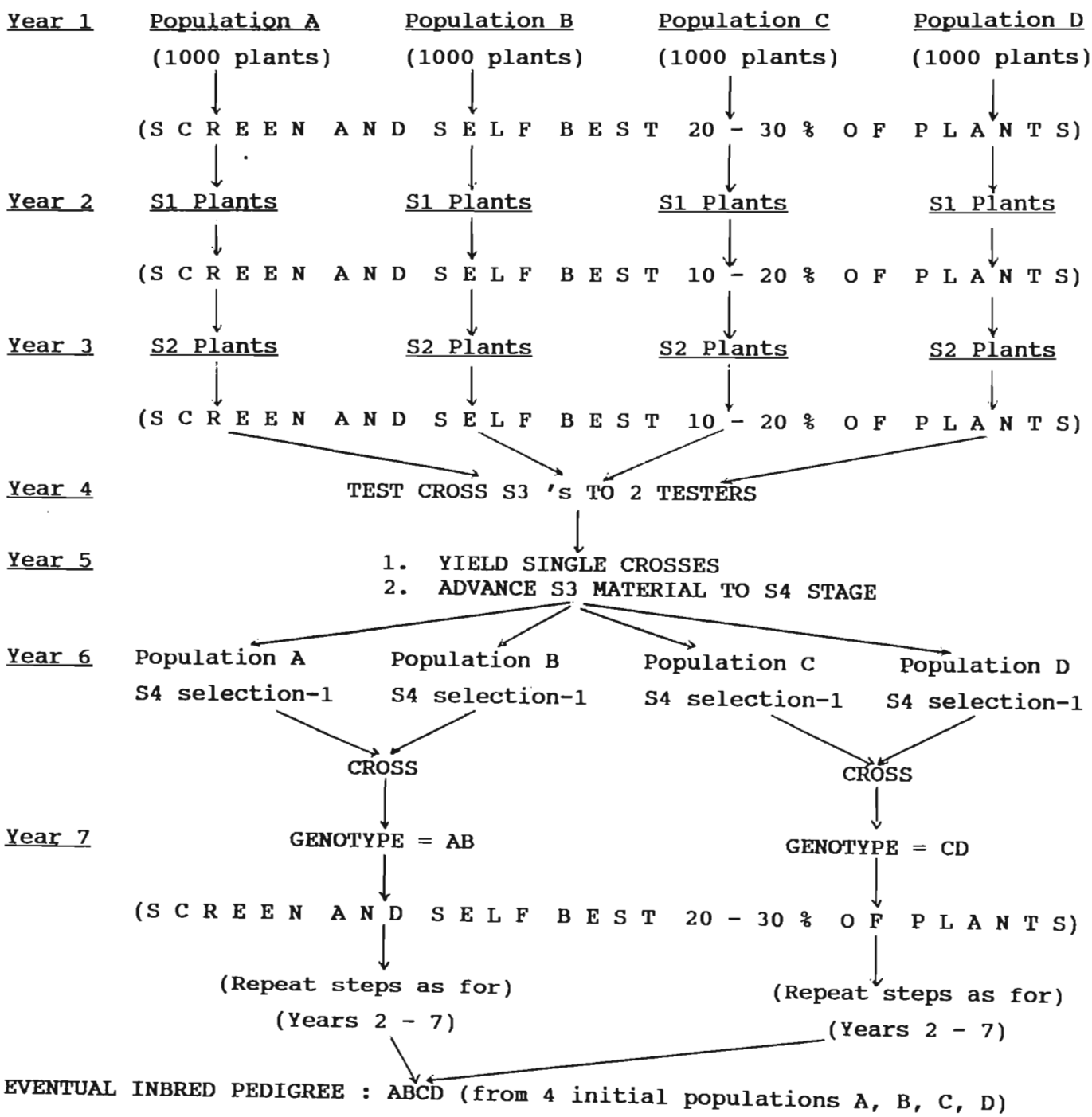
In the initial search for resistance to *B.fusca*, no resistance was discovered in any of several hundred elite yellow or white inbreds. These inbreds are used in the Pioneer maize programme to develop commercial hybrids, and practically all were found to be extremely susceptible. In 1978, out of 217 elite inbreds, 203 rated "5" for leaf damage, 11 rated "4", one rated "3" and only one rated "2". These latter two were obviously insufficient to begin an HPR programme, so the search moved onto intermediate and experimental inbreds. None showed any usable resistance. In addition, of 20 U.S. and South African elite populations screened, 3 U.S. populations showed some plants that were not as damaged as the other populations, but the leaf ratings were still a moderate "3". These plants were self pollinated in the hope that segregation for resistance would occur in the progeny. The selfing procedure explained previously was followed and eventually several intermediate inbreds, showing good levels of leaf resistance were obtained in 1983.

The path of germplasm development and utilization then took two directions. The one path went into hybrid development, which is described in 7.4. The other path went on to further utilization of these inbreds in resistance development. The best 10-20 % of the S4 intermediate inbreds which showed the most resistance and the best specific combining ability (s.c.a.) were then recombined

in 1983 by crossing them in all possible combinations in a recurrent selection programme. This term describes the continual infestation, evaluation and recombination over several years of the best segregants (S1, S2 or S3 stages) into new material that will hopefully give better resistance and better s.c.a. than the original germplasm. Some researchers recombine S1 material, but it was thought more satisfactory to recombine material at the S3 or S4 stage that had been confirmed as showing a resistant reaction as well as showing good s.c.a. for yield.

This procedure is shown in Fig. 20 where 4 original populations are utilized in a long term development of new resistant inbreds.

Fig. 20. Flow chart of development of resistant inbreds through recurrent selection.



The duration of the development of the inbred ABCD can be shortened in several ways. The S2's in year 3 can be screened and the best plants crossed without having to go to the S3 stage or test crossing the S3. By the time tasselling of the S2 stage occurs, the material will have been screened three times (S0, S1 and S2), and any plants still showing a resistant reaction can be crossed. The resultant F1 (S2 X S2) can be planted, screened and the best plants selfed. The process is repeated as above to get the best S2 plants. Again, with no test crossing (and therefore no information on s.c.a. for yield), the S2 plants from all 4 populations can be combined in year 6 to develop F1 plants from all populations. Selfing for 3-4 years will result in an inbred having been developed from the 4 populations. No data however is available on the yielding abilities of these short cycle selections, which will only be determined once they are test crossed.

It is also possible obviously to develop new germplasm by recurrent recombination from only 2 populations. In this case the development of an S3 plant from 2 populations would take only 10 years as in Fig.20, or 7 years in a recurrent system which does not evaluate the s.c.a. of the initial S3 selections. It is this latter system utilizing 2 populations, which has been predominantly used to develop resistance to *B.fusca* in S3 material which is then test crossed to ascertain s.c.a. for yield. In addition to the development of S3 material, the S3's are also crossed to each other to develop new populations. The full cycle of inbred and population development and their common procedures and backgrounds (see 7.2) is shown in Fig.21.

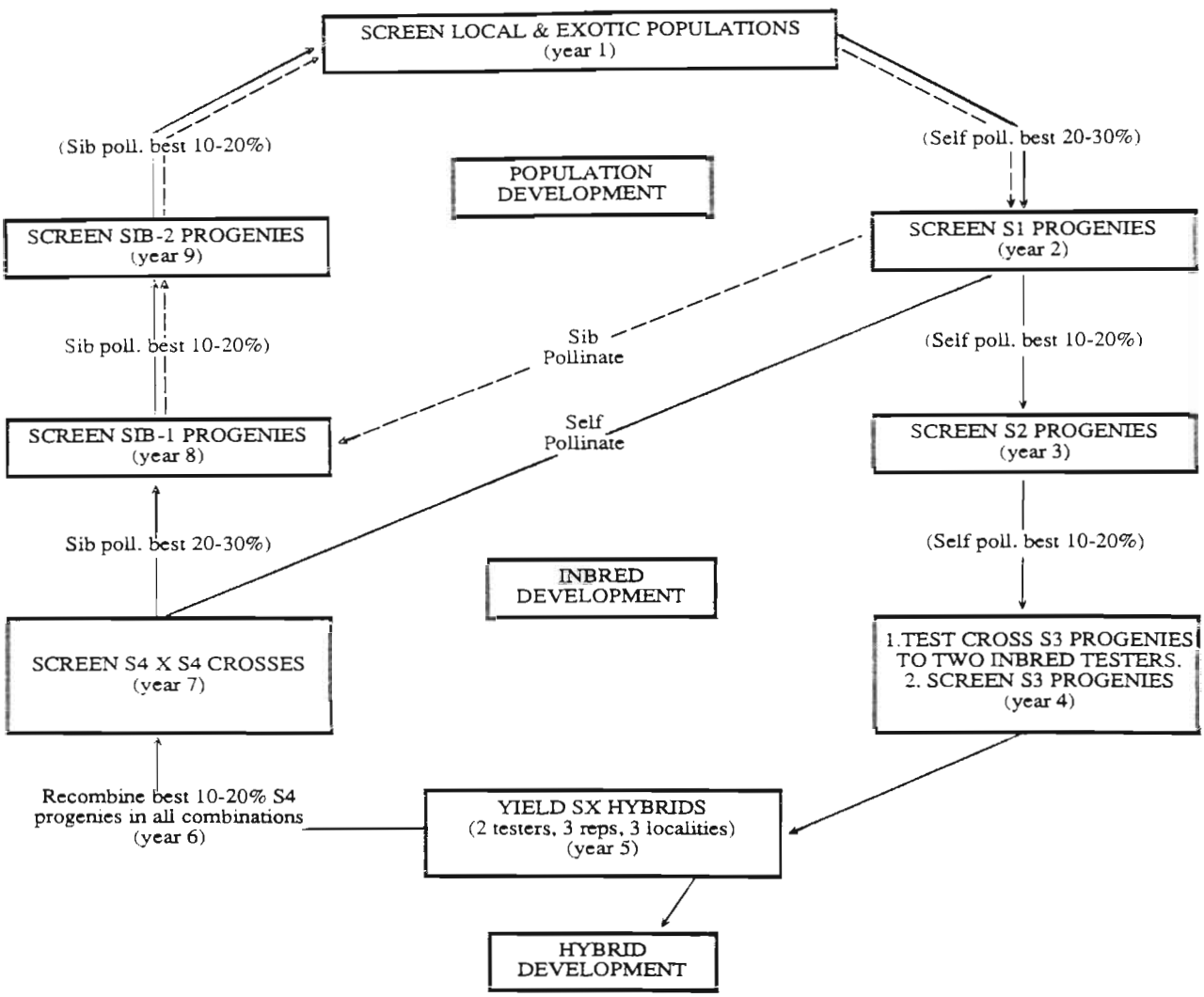


Fig.21. Development of stalk borer-resistant inbreds and populations.

7.2. POPULATION DEVELOPMENT

The development of new resistant heterozygous germplasm, from which new inbreds can be developed, forms an integral part of any HPR programme (see Fig.21). One of the best ways is to cross several disparate sources of resistance, in the hope that several new gene combinations will occur. The development of such populations was started in 1984, when the S4 x S4 crosses (see Fig.20) were evaluated in a separate field to that where inbred development took place.

These single crosses were infested and plants of the 20-30 % most resistant single crosses were then crossed with each other by sib-pollination. This process results in a thorough mixing of the germplasm and can result in extensive breaking and forming of gene linkages. The pollen from all selected plants was collected, mixed together and thoroughly shaken, and then placed onto the silks of all the plants. Seven separate groups of crosses were made up, constituting seven new populations. The sib-1 seed harvested is termed the F1 or S0 seed. The S0 seed was then planted out the following year (1985) for screening when segregation of resistant and susceptible responses occurred. A repeat of the sibbing of the best segregants was carried out to obtain the sib 2 seed. The sib 2 seed was planted in 1986, infested, and the most resistant plants sibbed again. The sib 3 seed that was harvested in 1987 was then planted in 1988, and infested. One hundred plants of these resistant plants as well as two susceptible populations (CSC and JLR) were rated in a non randomised observation trial (Table 7.1.)

Table 7.1. Leaf damage ratings (percentage of plants per rating group) of several populations artificially infested with *B.fusca*

	DAMAGE RATING				
POPULATION	1	2	3	4	5
ESB - 1	4	16	54	25	1
ESB - 2	15	35	29	19	2
ESB - 3	46	32	16	6	0
ESB - 4	52	30	18	0	0
ESB - 5	13	21	49	17	0
ESB - 6	48	40	12	0	0
ESB - 7	39	42	19	0	0
BSCB	0	2	16	49	33
CSC	0	0	0	27	73
JLR	0	0	0	4	96
SWCB	0	3	10	36	41

ESB -3, - 4, - 6 and - 7 showed the greatest number of resistant plants (rating 1 and 2). By alternate selfing and sibbing of all the 1 and 2 rated plants, the resistance of these populations will probably be improved further in yet another cycle of recurrent selection.

The population BSCB is an Iowa Corn Borer (*O. nubilalis*) resistant population,and SWCB is a population that was developed in Mississippi with resistance to the South Western Corn Borer (*D. grandiosella*). Both populations had some resistance to *B.fusca* but generally showed a susceptible reaction. The susceptible controls (CSC and JLR) showed extreme susceptibility in most plants.

The sibbing cycle was broken after 3 cycles by selfing to obtain homozygozity of any resistant genes. When this S1 seed was planted out in 1989, it was possible to detect the more resistant plants, and then to either continue selfing the plants (to develop new inbreds) or to return to a cycle of sib pollinations to reconstitute the same populations with greater resistance. New populations could also be developed after the full cycle of inbred development by sibbing the second cycle inbreds. These populations could then be used either as female parents in top cross hybrids (population female x inbred or single cross male) or as sources of new, hopefully more resistant, inbreds. It is obvious that many combinations of selfing and sibbing cycles can be utilized in developing resistance.

Once resistant inbreds have been developed, they can be used either to develop new, more resistant inbreds and populations, or as parents of resistant hybrids, or as donors of resistance onto other elite but susceptible inbreds. This can only be successful if the resistance is dominant with 1 or 2 genes involved. This is done by crossing a resistant inbred and a susceptible inbred, and extracting from the progeny the most resistant agronomically acceptable inbreds. Resistance can also be transferred through a backcrossing programme when only 1 or 2 genes are involved. If resistance is conditioned by several genes this procedure does not work, as reduced resistance generally occurs with each successive backcross.

Gene action conditioning resistance to most pests in maize appears to be additive (Maxwell and Jennings, 1980). This appears to be the case with *B. fusca*. Certain population improvement procedures, such as mass selection and various recurrent selection schemes have been found to be effective in accumulating desirable genes for resistance. In the HPR programme described in this thesis, recurrent selection as discussed in Fig. 20 has been found to produce the best resistance to *B.fusca*.

7.3. COMPARISON OF RESISTANT AND SUSCEPTIBLE INBREDS

Seven years after the HPR programme started, a selection of newly developed resistant inbreds were compared with several elite, but susceptible, inbreds. Among the susceptible inbreds were 3 inbreds which had been identified over several seasons (reported previously) as being the most resistant of all the elite inbreds. The objective of the following experiment was to determine whether the newer inbreds from the borer programme were more resistant than those elite inbreds.

(i) Materials and methods

Four inbreds that had been previously investigated in several experiments were compared with 9 newly developed inbreds. Of those 4 inbreds, 3 of them (F03, F23 and D57) were selected as showing a resistant reaction to *B.fusca*, and one (56) was chosen as a susceptible control. Of the 9 new inbreds, 5 had shown good leaf damage ratings in routine screening of company inbreds, 2 were intermediate in response, and 2 were included as susceptible controls. The experiment was planted on 3rd November 1986 in a randomized complete block design, with split plots and 3 replications. Each inbred row (whole plot) had 40 plants. The first 20 plants were utilized as two sub-plots, each containing 10 plants. These plants were destructively sampled 21 and 28 days after infestation, when larvae were removed for assessment of resistance on their development. The other 20 plants were used for assessment of leaf damage (after 21 and 28 days feeding), time of stem boring, days to tassel emergence, stem damage at harvest, height reduction and yield. All plots were separated from each other by a thickly sown row of plants. The plants were artificially infested 29 days post-emergence with approximately 19 larvae (mean of 18.6 larvae/plant over the whole trial).

(ii) Results and discussion

(a) Numbers of larvae/plant

There were highly significant differences ($P<0.01$) between the numbers of larvae recovered from each inbred. The numbers

recovered at the two sampling dates were also highly significantly different ($P<0.01$), as was the interaction between the two sources of variation.

Table 7.3.1. summarizes the results of the Analysis of Variance.

Table 7.3.1. Significance of mean numbers of larvae/plant recovered from 13 inbreds after 2 feeding periods (21 and 28 days feeding)

SOURCE OF VARIATION	F	F distribution values	
		5%	1%
Inbreds	239.45 **	2.18	3.03
Feeding periods	1093.75 **	18.51	98.50
Inbreds x feeding periods	30.33 **	2.18	3.03

C.V. % Whole Plots = 5.8 %
Sub-plots = 6.0 %

The effect of inbreds on numbers of larvae/plant

Table 7.3.2. Mean numbers of larvae/plant recovered from 13 inbreds averaged over 2 feeding periods

INBRED												
EB36	EB13	EB14	EB12	D57	F03	ES45	F23	EB37	EB32	ES41	ES42	56
0.68	1.30	1.76	2.03	2.60	3.43	3.55	4.06	4.28	4.45	5.46	6.23	6.45
a[1.]	b	c	c	d	e	e	f	fg	g	h	i	i

[1.] Means followed by the same letter are not significantly different at the 5% level

L.S.D. (5%) = 0.35

There was a very wide range in the numbers of larvae/plant

recovered from the inbreds. The numbers of larvae recovered here differed slightly from the numbers recovered from some of the same inbreds in 4.1.1.2. (Table 4.1.17), but the same trend was evident :

	Larvae / plant	
	Table 4.1.17	Table 7.3.2.
D57	2.27 a[1.]	2.60 d
F03	3.75 cd	3.43 e
F23	3.45 bc	4.06 f
56	4.27 de	6.45 i

[1.] **Means in each column followed by the same letters are not significantly different at the 5% level**

Where, in Table 4.1.17, D57 (with F08) showed the lowest numbers of larvae/plant (most resistant reaction of all inbreds), it now showed significantly more larvae/plant (2.60) than the top four ranked inbreds in Table 7.3.2. (range of 0.68 to 2.03 larvae/plant). These EB inbreds were developed over several years in the HPR programme, and show a most satisfactory, increased, level of antibiosis. Three other inbreds (ES45, EB37 and EB32) showed an intermediate response, and the inbred 56 was highly susceptible as expected.

<u>The effect of feeding periods on numbers of larvae/plant</u>

The mean numbers of larvae/plant removed from 13 inbreds after 21 and 28 days feeding were 4.23 and 2.89 respectively. These figures were significantly different (L.S.D. = 0.18, $P < 0.05$). This is discussed more fully under inbreds x feeding period interaction.

<u>The effect of the inbreds x feeding period interaction on numbers of larvae/plant (see Table 7.3.3)</u>.

Table 7.3.3. An interaction table showing the mean number of larvae/plant recovered from 13 inbreds after 21 days feeding

	INBRED												
FEEDING PERIOD	EB36	EB13	EB14	EB12	D57	F03	ES45	F23	EB37	EB32	ES41	ES42	56
21 Days	0.73 a[1]	1.63 b	2.43 c	2.50 cd	2.93 d	3.90 e	3.86 e	5.00 e	5.26 e	5.20 e	6.63 f	8.10 g	6.86 f
28 Days	0.63 l	0.97 lm	1.10 m	1.57 n	2.27 o	2.96 p	3.23 q	3.13 q	3.30 qr	3.70 r	4.30 s	4.36 s	6.03 t
	N.S.	*	*	*	*	*	*	*	*	*	*	*	*

[1] Means followed by the same letter are not significantly different at the 5% level

L.S.D. (5 %)	Main Effect	Interaction
Inbreds	0.35	0.35
Feeding periods	0.18	0.43

The number of larvae/plant after 21 days feeding ranged from 0.73 (EB36) to 8.10 (ES42) indicating that the inbreds showing the lower numbers obviously possessed a resistance mechanism which reduced numbers of larvae during the first 3 weeks of feeding. The excellent resistance of EB36 is clearly evident from the low numbers of larvae remaining after 21 days feeding.

As EB36 had such low numbers of larvae at the 21 day sampling, it was the only inbred that did not show a significant reduction in numbers of larvae over time. Obviously natural mortality caused a reduction in all inbreds, as the susceptible inbreds also showed significant reductions, but from a higher level. The imposition of barrier plants between the experimental inbreds prevented migration of larvae into the inbreds, as all inbreds (unlike those in 4.1.1.2.) showed a reduction in numbers of larvae. In 4.1.1.2, some inbreds showed an increase in numbers of larvae due to unimpeded migration.

(b) Larval mass

Table 7.3.4. summarizes the Analysis of Variance, comparing the mean larval mass recovered from 13 inbreds, over 2 feeding periods.

Table 7.3.4. **Significance of mean larval mass (mg) of larvae recovered from 13 inbreds after 2 feeding periods, 21 and 28 days feeding**

SOURCE OF VARIATION	F	F distribution values	
		5%	1%
Inbreds	326.48 **	2.18	3.03
Feeding periods	453.91 **	18.51	98.50
Inbreds x feeding periods	49.40 **	2.18	3.03

C.V. % Whole Plots = 5.7 %
Sub-plots = 10.0 %

There was a highly significant ($P<0.01$) difference between the mean larval mass of larvae recovered from each inbred, and from each sampling date. The interaction between the two variates was also highly significant ($P<0.01$).

The effect of inbreds on larval mass

Table 7.3.5. Mean larval mass (mg) of larvae recovered from 13 inbreds, averaged over 2 feeding periods

INBRED												
EB13	EB14	EB12	ES45	EB36	D57	EB32	EB37	ES41	ES42	56	F23	F03
8.93	9.80	9.98	10.34	13.75	15.27	16.84	16.99	20.21	21.69	29.78	40.30	46.61
a[1]	a	a	a	b	b	b	b	c	c	d	e	f

[1] Means followed by the same letter are not significantly different at the 5% level

L.S.D. (5 %) = 1.93

Comparison with previous data (Table 4.1.20) show the following larval mass recovered from some of these inbreds:

LARVAL MASS (mg)			
Table 4.1.20.		Table 7.3.5.	
D57	12.1 a[1]	D57	16.8 c
56	33.0 c	56	29.7 e
F23	46.1 d	F23	40.3 f
F03	61.7 e	F03	46.6 g

[1] Means in each column followed by the same letters are not significantly different at the 5% level

Although the values differ slightly, the ranking order prevails, with D57 showing the most resistant reaction and F03 showing the greatest susceptibility. In Table 7.3.5. however, there are 5 inbreds which show a significantly more resistant reaction than D57. These inbreds have a severe effect on larval mass gain. A comparison of the two resistance mechanisms (one affecting numbers and the other affecting larval growth) is shown in Table 7.3.6..

Table 7.3.6. Level of resistance to *B.fusca* in 13 inbreds, expressed as the number of larvae/plant and mean larval mass, averaged over 2 feeding periods

	NUMBERS OF	MEAN LARVAL	RESISTANCE AFFECTING :	
INBRED	LARVAE/PLANT	MASS (mg)	No's of larvae	Larval mass
EB36	0.68 a[1.]	13.75 n	Excellent Resist.	Intermed. Resist.
EB13	1.30 b	8.93 m	Excellent Resist.	Excellent Resist.
EB14	1.76 c	9.80 m	Excellent Resist.	Excellent Resist.
EB12	2.03 d	9.98 m	Excellent Resist.	Excellent Resist.
D57	2.60 e	15.27 o	Excellent Resist.	Intermed. Resist.
F03	3.43 f	46.61 s	Intermed. Resist.	Susceptible
ES45	3.55 f	10.34 m	Intermed. Resist.	Excellent Resist.
F23	4.06 g	40.30 r	Intermed. Resist.	Susceptible
EB37	4.28 gh	16.99 o	Intermed. Resist.	Intermed. Resist.
EB32	4.45 h	16.84 o	Intermed. Resist.	Intermed. Resist.
ES41	5.46 i	20.21 p	Susceptible	Intermed. Resist.
ES42	6.23 j	21.69 p	Susceptible	Intermed. Resist.
56	6.45 j	29.78 q	Susceptible	Intermed. Resist.

[1.] Means in columns followed by the same letter are not significantly different at the 5% level ($r = 0.36$, $P<0.05$)

Although the correlation between larval mass and numbers of larval/plant was significant, three of the inbreds did not perform as expected. EB36, which showed the most resistant reaction to numbers of larvae, showed intermediate resistance affecting mass gain, and did not retard larval growth as severely as expected. Both F03 and F23 showed an intermediate effect on numbers of larvae, but showed a highly susceptible effect on larval mass gain. ES45 showed an intermediate resistance affecting numbers of larvae, but excellent resistance affecting mass gain. These examples again support the hypothesis that there is no linkage of these two resistance mechanisms.

The effect of feeding period on larval mass

The mean larval mass removed from the plants after 21 and 28 days feeding were 12.94mg and 27.14mg per larvae respectively. This difference was highly significant ($P<0.01$) (L.S.D. = 2.83), with rapid mass gains being achieved during this period. Comparison with similar data in 4.1.1.2. (8.0mg and 55.2mg after 15 and 25 days feeding) illustrates the higher level of resistance in the genotypes in the present experiment, as the mean larval mass here was only 27.14mg, despite 3 days more feeding.

The effect of the inbreds x feeding periods interaction on larval mass

This interaction was highly significant ($P<0.01$).

Table 7.3.7. Mean larval mass (mg) of larvae recovered from 13 inbreds after 21 and 28 days feeding

FEEDING PERIOD	INBREDS												
	EB13	EB14	EB12	ES45	EB36	D57	EB32	EB37	ES41	ES42	56	F23	F03
21 Days	2.98 a[1]	5.30 ab	5.31 ab	6.82 b	13.43 c	7.92 b	12.69 c	7.46 b	15.39 c	19.89 d	21.83 d	21.95 d	27.23 e
28 Days	14.78 l	14.30 l	14.64 l	13.87 l	14.07 l	22.63 mn	20.99 m	26.52 n	25.04 n	23.48 mn	37.73 o	58.64 p	65.99 q
	*	*	*	*	N.S.	*	*	*	*	*	*	*	*

L.S.D. (5 %)	Main Effect	Interaction
Inbreds	1.93	3.53
Feeding periods	2.83	3.07

1. Means in rows followed by the same letter are not significantly different at the 5% level

With the exception of EB36, all inbreds showed significant increases in larval mass over time. The increases varied tremendously among inbreds. F23 and F03 were highly susceptible, especially when compared with ES42 and 56 which had similar 21 day masses. EB13, EB14 and EB12 all had larvae of a similar mass after 21 and 28 days (insignificantly different at $P<0.05$).

It is possible that some inbreds have a similar resistance mechanism effective for up to 21 days, but that the resistance has a longer residual in some inbreds. For example, EB14, EB12, ES45, D57 and EB37 also had non significantly different initial larval masses after 21 days, but significant differences occurred between the first 3 inbreds and D57 and EB37 after 28 days sampling : 14.30mg (l), 14.64mg (l) and 13.87mg (l) for the first 3 inbreds respectively, and 22.63mg (mn) and 26.52mg (n) for D57 and EB37. This longer acting resistance was also evident in EB36, EB32 and ES41, all of which had similar initial larval masses after 21 days feeding, but significantly different masses after 28 days (14.07 (l), 20.99 (m) and 25.04 (n) respectively). Even more dramatic were the significant differences evident between ES42, 56 and F23 which were initially similar in larval mass, but vastly different after an additional 7 days feeding : 23.48 (mn), 37.73 (o) and 58.64 (p) respectively. Obviously the longer lasting the resistance, the more effective as a pest management tool.

There were substantial differences in the larval mass recovered from each inbred at the 21 day sampling, with values ranging from 2.98mg per larvae to 27.23mg. Far greater differences occurred at the 28 day sampling, as the larvae feeding in the susceptible inbreds like F23 and F03 gained mass rapidly. The resistance in the better inbreds is probably effective for the entire duration of leaf feeding by larvae, as larvae generally feed for only 4-5 weeks before pupating. The quantitative expression of several genes acting additively is again demonstrated by the continuous range in larval masses recovered from all the inbreds. Also, the

level of resistance controlling mass gain exhibited by the more resistant inbreds is slightly better than any resistance recorded so far, but not nearly as improved as the resistance affecting larval numbers exhibited by the better inbreds in Table 7.3.3.

(c) Larval biomass/plant

Both mechanisms discussed above limit the numbers and development of larval feeding in leaf tissue. The resultant biomass/plant in each inbred and at each feeding period were highly significantly different ($P<0.01$).

The interaction was also highly significant ($P<0.01$).

Table 7.3.8. Significance of mean larval biomass/plant recorded in 13 inbreds after 2 feeding periods, 21 and 28 days feeding

SOURCE OF VARIATION	F	F distribution values	
		5%	1%
Inbreds	345.03 **	2.18	3.03
Feeding periods	2610.21 **	18.51	98.50
Inbreds x feeding periods	31.29 **	2.18	3.03

C.V. % Whole Plots = 7.7 %
Sub-plots = 11.5 %

The effect of feeding period on larval biomass/plant

There were highly significant ($P<0.01$) differences between the mean larval biomass/plant removed from the inbreds after 21 and 28 days feeding (63.44mg/plant and 87.64mg/plant respectively; L.S.D. = 2.04).

The effect of inbreds on larval biomass

The highly significant ($P<0.01$) differences between inbreds with regard to the mean larval biomass removed from all plants are shown in Table 7.3.9.

Table 7.3.9. Mean larval biomass/plant (mg) recovered from each of 13 inbreds, averaged over 2 feeding periods

INBRED												
EB36	EB13	EB14	EB12	ES45	D57	EB37	EB32	ES41	ES42	F23	F03	56
9.35	9.56	14.40	18.17	35.77	37.39	63.33	72.09	104.53	131.80	146.31	150.65	188.70
a[1.]	a	a	a	b	b	c	c	d	e	f	f	g

[1.] Means followed by the same letter are not significantly different at the 5% level

L.S.D = 9.77

In 4.1.1.2. (Table 4.1.24), the lowest larval biomass/plant was 24.6mg, averaged over 15 and 25 days feeding (D57 had the second lowest value of 29.1mg/plant). The figures for larval mass were also higher for most inbreds in that experiment than in this one, despite the fact that in 4.1.1.2. the larvae fed for 3 days less. A comparison of the larval biomass in both experiments is shown below:

	Larval biomass (mg)	
	Table 4.1.24.	Table 7.3.9.
D57	29.1 a[1.]	37.39 b
F23	162.9 d	146.31 f
F03	246.1 e	150.65 f
56	149.2 cd	188.70 g

[1.] Means in each column followed by the same letter are not significantly different at the 5% level

With the exception of F03 (which had the highest larval mass of these inbreds in 4.1.1.2.), fairly good correlation over seasons occurred. In table 7.3.9. there were very low biomasses recorded from several inbreds, with 4 of them showing a significantly lower larval biomass than that recorded for D57. The high level of resistance in these inbreds is thus most apparent.

<u>The effect of inbreds x feeding periods on larval biomass</u>

This interaction is shown in Table 7.3.10.

Table 7.3.10 Mean larval biomass/plant (mg) of larvae recovered from 13 inbreds after 21 and 28 days feeding

FEEDING PERIOD	INBRED												
	EB36	EB13	EB14	EB12	ES45	D57	EB37	EB32	ES41	ES42	F23	F03	56
21 Days	9.80 a[1]	4.90 a	12.92 ab	13.26 ab	26.34 bc	23.24 bc	39.53 c	65.94 d	102.00 e	161.14 f	109.72 e	106.06 e	149.89 f
28 Days	8.90 l	14.23 l	15.89 l	23.08 l	45.20 m	51.55 m	87.13 n	78.23 n	107.06 o	102.47 o	182.89 p	195.23 p	227.51 q
	N.S.	N.S.	N.S.	N.S.	*	*	*	N.S.	N.S.	N.S.	*	*	*

[1] Means followed by the same letter are not significantly different at the 5% level

L.S.D. (5 %)	Main Effect	Interaction
Inbreds	9.77	14.12
Feeding periods	2.04	14.24

The values and inbred ranks follow those in Table 7.3.3. (numbers) and Table 7.3.7. (mass) fairly closely. The reasons for the significance, or lack of it, are adequately discussed above. There were very large significant differences between the more resistant inbreds and the most susceptible inbreds. The effect of the differences in larval biomass on the plants is discussed next.

(d) Leaf damage

The mean leaf damage ratings taken on the inbreds were highly significantly ($P<0.01$) different, but not at the two sampling times. The mean leaf damage rating was 2.06 after 21 days feeding and 2.26 after 28 days feeding (N.S.; L.S.D. = 0.26). The interaction was highly significant.

Table 7.3.11. Significance of the mean leaf damage ratings from 13 inbreds, averaged over 2 feeding periods (21 and 28 days feeding)

SOURCE OF VARIATION	F	F distribution values	
		5%	1%
Inbreds	263.13 **	2.18	3.03
Feeding periods	10.61 N.S.	18.51	98.50
Inbreds x feeding periods	18.91 **	2.18	3.03

C.V. % Whole Plots = 4.7 %
Sub-plots = 5.3 %

The effect of inbreds on leaf damage

Table 7.3.12. Leaf damage ratings on 13 inbreds averaged over 2 periods, 21 and 28 days feeding

INBRED												
EB13	EB12	EB14	EB36	F03	ES45	EB37	F23	D57	EB32	ES42	ES41	56
1.03 a[1.]	1.25 b	1.26 b	1.26 b	1.75 c	1.76 c	1.98 d	1.98 d	2.35 e	2.61 f	3.38 g	3.56 h	3.88 i

[1.] Means followed by the same letter are not significantly different at the 5% level
L.S.D. (5 %) = 0.17

Not as great a range in leaf damage occurred as expected, although the damage differences were significant. In 5.2. (Table 5.11.) some of these inbreds were infested and leaf damage was rated after 24 days feeding:

	Mean leaf damage ratings	
	Table 5.11	Table 7.3.12
F23	1.84 a	1.98 c
F03	1.99 ab	1.75 d
D57	2.01 ab	2.35 e
56	4.00 e	3.88 g

The same trend was evident with very similar results over trials. In Table 7.3.12. the lower leaf damage ratings of EB13 (1.03), EB12 (1.25), EB14 (1.26) and EB 36 (1.26) show good improvement in breeding for leaf resistance. As expected, ES42 and ES41, inbreds developed in a maize streak virus programme, showed a susceptible reaction, as did the control inbred 56.

The effect of inbreds x feeding periods on leaf damage

Although the interaction was significant, the increase in leaf damage over time was slight. The range of values for the 21 and 28 day ratings did not vary much from the mean, and will therefore not be discussed.

The effect of the two resistance mechanisms on leaf damage

The relative contributions of the two resistance mechanisms and the final leaf damage ratings are tabulated below :

	RANKINGS ACCORDING TO :			
INBRED[1.]	Leaf damage Rating/Plant	Numbers of Larvae/plant	Mass of Larvae	Larval Biomass
EB13	1 a	2 b	1 a	2 a
EB12	2 b	4 d	3 a	4 a
EB14	3 b	3 c	2 a	3 a
EB36	4 b	1 a	5 b	1 a
F03	5 c	6 f	13 g	12 f
ES45	6 c	7 f	4 a	5 b
EB37	7 d	9 gh	8 c	7 c
F23	8 d	8 g	12 f	11 f
D57	9 e	5 e	6 c	6 b
EB32	10 f	10 h	7 c	8 c
ES42	11 g	12 j	10 d	10 e
ES41	12 h	11 i	9 d	9 d
56	13 i	13 j	11 e	13 g

[1.] Inbreds arranged in order of increasing leaf damage rating (see Table 7.3.12).

Correlation matrix:

	1	2	3	4
1. Leaf damage	1			
2. Numbers	+0.89 **	1		
3. Mass	+0.47 *	+0.48 *	1	
4. Biomass	+0.70 **	+0.79 **	+0.82 **	1

5 % r = 0.46
1 % r = 0.63

With the exception of EB36 (larval mass), the four top - ranked inbreds for leaf damage were the four top - ranked for numbers of larvae/plant, mean larval mass and biomass of larvae/plant. The 3 bottom ranked inbreds for leaf damage were the inbreds that showed lack of resistance mechanisms affecting numbers and mass. The middle group changed slightly depending on the attribute being recorded. The strange phenomenon of F03 and F23 showing low leaf damage but no resistance mechanisms controlling numbers of larvae or mass gain has been explained in 4.1.1.2. It was surmised that the nutritional status of these inbreds was so high that the larvae managed to gain mass rapidly without consuming large amounts of leaf tissue. Again it is evident that assessment of leaf damage, by rating the extent of feeding after about 4 weeks feeding, is a rapid and efficient indicator of resistance in plants under attack by larvae.

(e) Stem damage

The Analysis of Variance, comparing the mean stem damage ratings at harvest over all inbreds is summarized in Table 7.3.13.

Table 7.3.13. Significance of mean stem damage ratings of 13 inbreds, recorded at harvest

SOURCE OF VARIATION	F	F distribution values	
		5%	1%
Hybrids	65.23 **	2.18	3.03

C.V. % Plots = 12.4 %

These highly significant differences (P<0.01) are presented in Table 7.3.14.

Table 7.3.14. Mean stem damage ratings at harvest of 13 inbreds recorded on a 1-9 scale (1= minimal; 9 = severe)

INBRED												
EB12	EB36	EB14	EB37	EB13	ES45	D57	ES41	F23	EB32	F03	ES42	56
0.73 a[1.]	1.50 ab	1.86 b	1.96 b	2.03 b	2.26 b	3.56 c	5.00 d	5.80 de	6.00 e	6.50 e	6.50 e	6.56 e

[1.] Means followed by the same letter are not significantly different at the 5% level

L.S.D. (5 %) = 0.98

Comparison with data in Table 5.14 shows similar levels of stem damage over seasons, except for F03 :

INBRED	Mean stem damage ratings	
	Table 5.14	Table 7.3.14
F03	3.54 a	6.50 e
D57	3.73 ab	3.56 c
F23	6.45 de	5.80 de
56	6.74 e	6.56 e

The improvement in resistance to stem damage, which is expressed by reduced stunting, shown by many of the EB inbreds in table 7.3.14, and in particular by EB12 (mean stem damage rating of 0.73), is remarkable (Plate 17).

(f) Stunting

The effect that stalk borer larvae had on final plant height is shown in Table 7.3.15.

Table 7.3.15. Significance of mean stunting ratings recorded at post pollen shed of 13 inbreds

SOURCE OF VARIATION	F	F distribution values	
		5%	1%
Inbreds	18.54 **	2.18	3.03

C.V. % Plots = 13.9 %

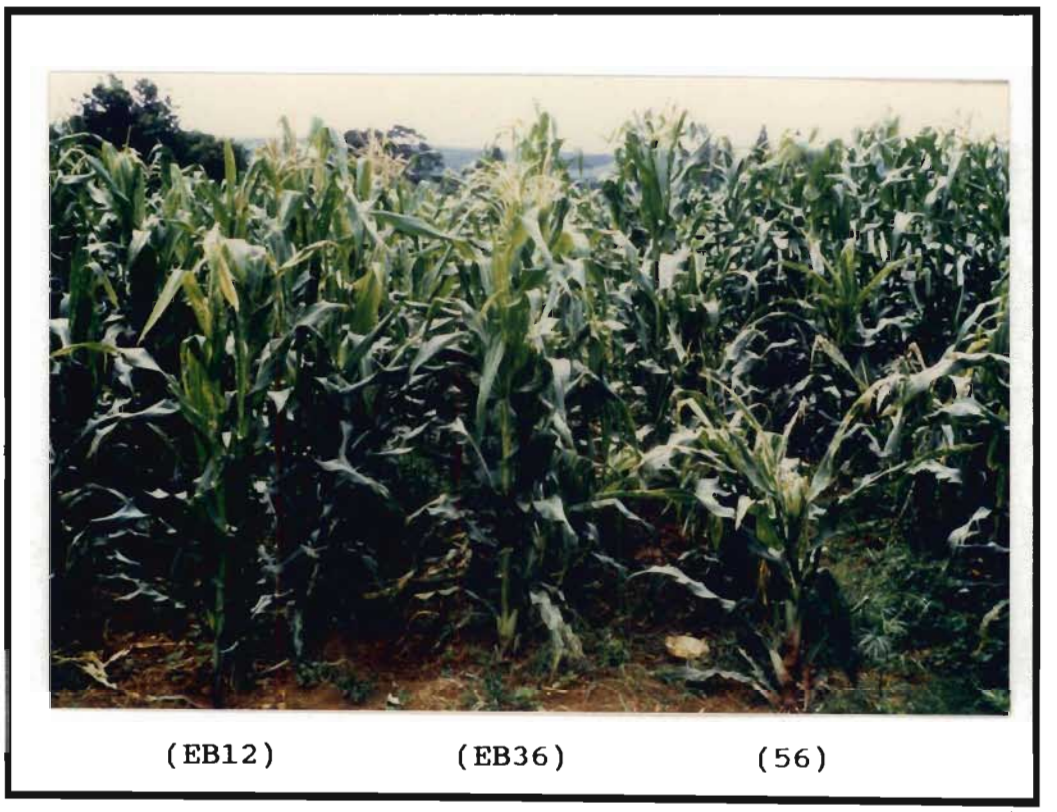


Plate 17. Resistant inbreds (EB12 & EB36) planted next to a highly susceptible inbred (56).

Table 7.3.16. Mean stunting ratings of 13 inbreds recorded at post pollen shed

INBRED												
EB12	EB36	EB37	EB14	EB13	ES45	D57	F23	EB32	ES41	ES42	F03	56
1.00	1.20	1.43	1.65	1.78	2.14	2.30	2.78	3.24	4.16	4.84	5.00	5.00
a[1]	ab	bc	c	cd	de	e	f	g	h	i	i	i

[1] Means followed by the same letter are not significantly different at the 5% level

L.S.D. (5 %) = 0.37

There were significant differences evident between the inbreds. Correlation over seasons was also good, except for F03, which was most severely stunted due to extensive stem damage. The stem boring had obviously occurred earlier here than in the experiment described in Chapter 5.

	Mean stunting ratings	
	Table 5.13	Table 7.3.15
F03	2.25 b	5.00 i
D57	2.25 b	2.30 e
F23	3.00 bc	2.78 f
56	5.00 e	5.00 i

The benefit of increased resistance in inbreds like EB12, EB36 and EB37 is very clearly demonstrated.

(g) Yield

The effect on yield, of larvae feeding in leaf and stem tissue of the inbreds, was investigated by comparing the mean yields of infested and uninfested plants :

Table 7.3.17. Significance of percentage yield loss of 13 inbreds after uncontrolled feeding by stalk borer larvae

SOURCE OF VARIATION	F	F distribution values	
		5%	1%
Inbreds	15.79 **	2.18	3.03

C.V. % Plots = 13.6 %

Table 7.3.18. Mean grain yields (g/plant) of 13 inbreds, recorded from infested and uninfested plots

INBRED	MEAN YIELD / PLANT (g)		% YIELD	
	UNINFESTED	INFESTED	REDUCTION	
EB36	73.70	65.67	10.89	a[1.]
EB13	52.27	44.43	14.99	a
EB12	105.00	83.67	20.31	a
ES45	59.90	36.50	39.06	b
EB14	88.93	53.30	40.06	b
EB37	57.63	34.27	40.53	b
F23	84.24	26.95	68.00	c
D57	97.07	27.60	71.56	cd
ES41	102.60	26.32	74.34	cde
F03	36.22	8.23	77.28	de
EB32	118.80	22.00	81.48	e
56	133.00	22.67	82.95	e
ES42	105.73	14.80	86.00	e

[1.] Means followed by the same letter are not significantly different at the 5% level

L.S.D. (5 %) = 9.42

The relative rankings of the inbreds common to Table 5.18 and Table 7.3.17 were similar :

	% Yield loss	
	Table 5.18	Table 7.3.17
D57	53.36 abc	71.56 cd
F03	60.36 bc	77.28 de
F23	85.42 de	68.00 c
56	100.00 e	82.95 e

The lowest yield loss in Table 5.22 was 38.57% for D54. The results from Table 7.3.17 show a much improved reduction in yield loss with EB 36 showing the smallest reduction of only 10.89%.

As has been discussed previously, yield loss is caused primarily by extensive stem feeding, which is itself controlled by a complex interaction of time of infestation, larval populations, days to flowering, time of stem boring, plant height, and yield potential of the cultivar. The analysis of correlation showed the following relationship between all attributes :

Table 7.3.19. Correlation matrix of various attributes of 13 inbreds

	1	2	3	4
1. % Yield loss	1			
2. Stem damage ratings	+0.74 **	1		
3. Stunting ratings	+0.61 **	+0.64 **	1	
4. Leaf damage ratings	+0.50 *	+0.49 *	+0.48 *	1

5 % r = 0.43
1 % r = 0.59

All attributes showed significant correlation, with the strongest correlations, as in previous experiments, occurring between stem damage and yield loss, and stem damage and stunting.

In conclusion, it can be stated that the development of inbreds resistant to *B. fusca* has so far been successful. The three resistance mechanisms which affected larval growth and numbers of larvae were improved considerably through a system of recurrent recombination. The inbreds investigated in this experiment were the first group of inbreds developed in the programme, and it is hoped that the continuing programme will result in inbreds and populations that are more resistant than these. These inbreds are currently being assessed in hybrid combinations, the background of which is discussed in 7.4.

7.4. THE DEVELOPMENT OF STALK BORER-RESISTANT HYBRIDS

Once resistant inbreds have been developed, they are placed into a testing system that evaluates their specific combining ability with other inbreds for many agronomic traits apart from resistance to *B. fusca*:

Fig. 22. Development of borer-resistant hybrids

(following on Fig. 21)

Year 6

1. Test cross best 10-20% S3 progenies ("Initials") to 4 inbred testers = SX hybrids
2. Screen same S3 progenies and self to S4

Year 7

1. Yield & screen SX hybrids (4 Testers,3 reps,3 localities)
2. Screen S4 progenies & self to S5

Year 8

1. Test cross best 10-20% S5 progenies ("Intermediates") to 10 inbred testers
2. Bulk up all S5 progenies (500g)

Year 9

Yield SX hybrids (10 Testers, 3 reps, 3 localities)

Year 10

Make hybrids from best resistant and agronomic inbreds
(SX = 1 year ; DX = 2 years)

Year 11

Yield and screen hybrids.

Year 12

Commence parent seed production of selected resistant hybrids

Year 13

Make up final hybrids in sufficient quantities for commercial sale

Year 14

Commence commercial sales.

It is not possible to ascertain visually whether a plant showing a resistant reaction contains dominant, recessive or additive genes for resistance, nor is it possible to determine how many genes are involved.It is only once an inbred is crossed with another plant, and the progeny infested and the resistance assessed, that one can hypothesise as to the number of genes involved, and as to the inheritance of resistance. It is quite possible, as occurred with this study on *B.fusca*, to not have any knowledge of the number of genes involved in resistance and yet to still have success in developing resistant inbreds. However, in the making up of hybrids, it is necessary to know whether the resistance is additive, dominant or recessive. The mode of inheritance of resistance will determine whether a hybrid requires 1, 2, 3, or 4 resistant inbreds for resistance to be expressed. Most commercial hybrids in South Africa contain 3 or 4 inbreds (A, B, C and D) as parents. A three parent hybrid would be represented as a single cross female (A x B) crossed onto an inbred male (C). This hybrid would be represented as A x B / C.

A 4 parent, or double cross hybrid would be represented as a single cross female (A x B) crossed onto a single cross male (C x D). This hybrid would be represented as A x B / C x D.

The various possibilities of how resistant hybrids would be constituted are discussed :

1. <u>Single gene dominance</u>

This is the easiest form of resistance to utilize in any hybrid, and can confer resistance in the heterozygous form. It would be a rare occurrence in HPR work if the first few resistant hybrids produced contained only resistant inbreds. Initially, only 1 or 2 resistant inbreds are combined with elite, but susceptible, inbreds. It is therefore necessary to know on which side of the hybrid combination the resistant inbred/s must be placed. Several examples will illustrate the correct placement of one or two resistant inbreds in 3 or 4 parent hybrids (Resistance is represented R, with the susceptible allele represented by r) :

(i) 3 Parent hybrids A x B / C using only 1 resistant inbred (RR):

There are two possibilities:

(a) Parents :(RR x rr) x rr
Gametes :(R and r) x r
F1 Hybrid plants :Rr and rr (50 % resistant / 50 % susceptible)

or

(b) Parents :(rr x rr) x RR
Gametes : r x R

F1 Hybrid plants : Rr (100 % resistant)

It can be seen that in (a), if the resistant inbred is placed in the single cross on the female side in combination with a susceptible male inbred, then the resulting hybrid will have 50 % of the plants resistant and 50 % susceptible. However if the male inbred is the resistant germplasm (as in (b)), then the resultant hybrid will be 100 % resistant. This would enable two susceptible but elite inbreds to be used in conjunction with a resistant, but possibly intermediate potential, inbred. If two resistant inbreds are used in a 3 parent hybrid, it is obvious that the dominance on either side would produce a resistant hybrid.

(ii) 4 Parent or double cross A x B / C x D using only 1 resistant inbred :

Parents :(RR x rr) x (rr x rr)
Gametes : R and r x r

F1 Hybrid plants :Rr and rr (50% resistant / 50% susceptible)

Obviously 1 resistant inbred in a double cross hybrid is insufficient to give total resistance. The damage to the susceptible plants would invalidate any claim to marketing a

resistant hybrid.

(iii) Double cross hybrids with 2 resistant inbreds :

There are two possibilities:

(a) Parents :(RR x rr) x (RR x rr)

Gametes :R and r x R and r

F1 Hybrid plants :RR ; Rr; rr (25 %; 50 %; 25 %)

All plants containing R will be resistant (75%), possibly allowing the claim of resistance to be applied in marketing such a hybrid.

(b) Parents :(RR x RR) x (rr x rr)

Gametes : R x r

F1 Hybrid plants : Rr

Such a hybrid would give 100 % resistant plants, and this configuration of inbreds would be the best arrangement.

(iv) Double cross hybrid with 3 resistant inbreds:

Any such hybrid would have the resistance expressed in all plants.

2. Recessive resistance

If one assesses the use of recessive resistance, the make up of a hybrid is even more important. To be effective, recessive resistance must be in a homozygous form. Examples are given of the correct placement in a hybrid of an inbred with recessive resistance (rr) :

(i) 3 Parent hybrid A x B / C with 1 resistant inbred :

There are two possibilities:

(a) Parents :(RR x RR) x (rr)

(Susc.) (Resist.)

Gametes : R x r

F1 Hybrid plants : Rr (All plants will be susceptible)

(b) Parents :(RR x rr) x (RR)
(Susc.) (Susc.)
Gametes : R and r x R
F1 Hybrid plants : RR and Rr (All plants will be susceptible)

Obviously one resistant hybrid with recessive resistance in a three parent hybrid is insufficient to produce a resistant hybrid, no matter where it is placed in the hybrid combination.

(ii) 3 Parent hybrid A x B / C with 2 resistant inbreds

There are two possibilities:

(a) Parents :(RR x rr) x rr
(Susc.) (Resist.)
Gametes : R and r x r
F1 Hybrid plants : Rr and rr (50 % resistant / 50 % susceptible)

(b) Parents :(rr x rr) x (RR)
(Resist.) (Susc.)
Gametes : r x R
F1 Hybrid plants : Rr (All plants would be susceptible)

With recessive resistance, a hybrid would have to have all 3 inbreds with the resistant genes for effective resistance to occur. This severely limits the use of recessive resistance in the development of stalk borer-resistant hybrids.

(iii) Double cross hybrids with 1 resistant inbred:

Any such hybrid would have all plants susceptible.

(iv) Double cross hybrids with 2 resistant inbreds:

There are two possibilities:

(a) With 2 resistant inbreds, 25 % of the plants would show a resistant reaction if each parent had a recessive resistant parent :

Parents :(RR x rr) x (RR x rr)

Gametes : Rr x Rr

F1 Hybrid plants : RR; Rr; rr (75 % Susceptible / 25 % Resistant)

(b) If the two resistant inbreds were on one side of the hybrid, all the plants would be susceptible in the final hybrid :

Parents :(RR x RR) x (rr x rr)

Gametes : R x r

F1 Hybrid plants : Rr (100 % Susceptible)

(v) Hybrids with 3 resistant inbreds:

Any such hybrid would have 50 % of the plants resistant, and 50% would be susceptible:

Parents :(RR x rr) x (rr x rr)

Gametes : R and r x r

F1 Hybrid plants : Rr and rr (50 % Resistant / 50 % Susceptible)

(vi) Only with 4 resistant (recessive) inbreds, would a totally resistant hybrid result.

It is evident that recessive resistance, which in itself is not easy to identify, would not be much use in a HPR programme.

3. Additive resistance

This is the most common form of resistance found in insect HPR programmes. It is evident, from preliminary analyses of the results of crosses involving resistant and susceptible inbreds, that the gene action conditioning resistance to *B.fusca* is of the additive type. No calculation has been made as to the number of genes involved. With additive resistance, the more

genes involved, the more difficult it is to develop inbreds or populations with good resistance to the insect. It is also more difficult to get all the required genes into hybrid combination, especially when double cross hybrids with 4 inbred parents are required for the market. The fewer the inbreds involved in the hybrid parentage, the easier it is to get any attribute into a hybrid, provided all the required genes are carried by those inbreds. The permutations that are possible where more than 2 genes are involved preclude any illustration of how the inbreds should be arranged in making up a hybrid. This is especially the case where each mechanism is characterized by different genes. Of, say, 4 genes required for the additive resistance, some could show a dominant reaction, and the others a recessive reaction.

The only way to ascertain which inbreds provide sufficient resistance is to screen them in combination with other tester inbreds. Some of these tester inbreds should be resistant in order to pick up specific combining ability for resistance. In such cases, the resultant single cross hybrid will show greater resistance than either of the two inbreds. Some of the tester inbreds should also be susceptible in order to identify dominance of the resistant inbred. In such cases, the single cross hybrid will not show a mid-parent (intermediate) reaction (as expected in an additive system), but will show the same level of resistance as the resistant inbred.

The above examples illustrate the necessity of correct placement of the resistant inbreds in any crosses. What complicates the matter further is that one has to take cognisance of other attributes in making up hybrids. Yield, disease resistance and lodging resistance are a few of the attributes that are profoundly affected by changing around the conformation of a hybrid. It is only by lengthy testing of many inbreds in many combinations over several seasons that a confident assessment can be made as to the stability of resistance and the potential of the hybrid in competition with other elite hybrids on the open market.

7.5 ASSESSMENT OF POTENTIAL STALK BORER-RESISTANT HYBRIDS

In the 1987/88 season, several single cross, three way cross and double cross hybrids were made, utilising inbreds which showed a high level of borer resistance. Yield trials were planted at two sites, Greytown and Delmas, and a separate, unrandomised, single replication was planted, infested and rated at Greytown. Several susceptible commercial hybrids were included in the trial for comparison of susceptibility and yield potential. The objective was to evaluate the relative yield potentials and resistance levels of all hybrids, in order to assess the feasibility of the development of commercial borer-resistant hybrids.

(i) Materials and methods

The majority of yield trials conducted by Pioneer Seed Company are triple lattice designs, planted at numerous locations in South Africa. A trial of 42 hybrids was randomised accordingly and included 9 commercial, but susceptible, hybrids as controls. There were 8 double cross hybrids made up with some of the more borer-resistant inbreds developed over the past years in the borer programme, 8 three way hybrids consisting of susceptible single cross females crossed to resistant inbred males, 9 resistant inbred x resistant inbred single cross hybrids, and 8 resistant x susceptible inbred single cross hybrids. Routine chemical control of *B. fusca* was applied to the uninfested rows during the season.

The trials were planted as 20 plant rows, 2 row plots/hybrid with 3 replications in early October 1988. The two sites were at the Greytown and Delmas Research Stations, and they were yielded in April 1989. Yields were converted to 12,5% moisture and presented in Tonnes/ha, and expressed as a percentage of the mean of all entries (i.e. mean percentage = 100%). The data were analyzed on a Sperry 5000-30, using an in-house statistical programme written in Pascal. An Analysis of Covariance was carried out on yield and stand, with adjustments for blocks and regression where necessary.

In addition to the yield trial, a single replicate of 20 plants per hybrid was planted in Greytown on 8th November 1988 and infested on 14th December 1988 with a mean of 21.2 larvae/plant. Leaf damage was rated on a single plant basis, on a 1-5 scale, 25 days later. The data are presented in Table 7.5.1..

Table 7.5.1. Mean leaf damage ratings, yield potential (uninfested), grain moisture at harvest, and prolificacy of 42 hybrids grown at 2 locations

RANK [1]		HYBRID	PARENT [2] RES./SUSC.	GRAIN COLOUR [3]	LEAF DAMAGE RATING	MEAN REL. YIELD [4] (%)	ACTUAL YIELD (T/HA) GREYTOWN	DELMAS	GRAIN MOISTURE %	PROLIF. INDEX [5]
1	*	PNR 6556	4S	Y	5	146.25	10.36	8.90	18.25	1.63
2	*	PNR 6462	4S	Y	5	137.48	10.47	7.72	17.45	1.45
3	*	PNR 6549	4S	W	5	130.01	9.51	7.64	17.45	1.44
4	*	PNR 6363	4S	W	5	126.10	9.21	7.43	15.85	1.59
5		EB56xK10	RxS	Y	3	123.73	10.65	5.86	18.90	1.07
6		K10xM24/EB37	2S/R	Y	3	123.66	8.77	7.51	15.45	1.42
7	*	PNR 6463	4S	W	5	120.69	9.03	6.91	17.90	1.32
8	*	PNR 6429	4S	W	5	116.08	8.02	7.24	16.90	1.29
9		EB62xK10	RxS	Y	2	113.25	7.81	7.07	15.45	1.46
10		EB58xM13	RxS	Y	4	112.59	8.39	6.48	20.80	1.38
11		EB23xEB24	RxR	Y	3	111.61	8.11	6.60	17.45	1.62
12	*	PNR 6334	4S	Y	5	110.29	7.92	6.61	14.45	1.49
13		K12x59/EB12	2S/R	W	2	109.87	7.50	6.93	18.30	1.18
14		B22xM59/EB12	2S/R	W	2	109.85	6.46	7.85	16.40	1.40
15		EB59xM13	RxS	W	2	109.81	8.55	5.99	18.00	1.34
16		EB20xK11	RxS	Y	2	108.17	7.41	6.80	15.65	1.49
17		EB58xK10	RxS	Y	2	108.14	6.94	7.21	15.55	1.58
18		J47xM59/EB12	2S/R	W	3	101.94	6.73	6.63	17.40	1.15
19		EB33xEB37/EB23xEB24	4R	Y	4	100.46	7.33	5.92	17.45	1.25
20		EB27xEB30/EB23xEB24	4R	Y	3	100.30	5.99	7.09	15.85	1.21
21		K12xM59/EB13	2S/R	W	2	99.97	6.89	6.25	16.00	1.35
22		EB23xEB36	2R	Y	1	99.72	7.24	5.91	17.25	1.29
23		EB23xEB33/EB27xEB24	4R	Y	4	96.69	6.48	6.21	16.40	1.15
24	*	PNR 542	4S	Y	5	96.41	6.61	6.05	16.90	1.41
25		EB56xM13	RxS	Y	3	96.25	6.87	5.81	16.20	1.40
26		EB23xEB30	2R	Y	1	95.61	6.78	5.80	13.30	1.19
27		EB54xM13	RxS	Y	3	94.44	7.36	5.15	14.95	2.06
28		K037xM59/EB13	2S/R	W	4	94.29	6.95	5.49	17.50	1.15
29		K80xM24/EB36	2S/R	Y	3	94.26	6.95	5.49	16.20	1.18
30		EB27xEB36/EB23xEB24	4R	Y	3	92.98	5.52	6.60	13.85	1.17
31	*	PNR 394	4S	Y	5	90.00	6.67	5.21	15.90	1.26
32		EB23xEB28/EB33xEB37	4R	Y	4	87.54	6.23	5.30	16.15	1.19
33		EB23xEB38	2R	Y	1	86.18	5.61	5.68	13.85	1.11
34		EB24xEB27	2R	Y	2	86.16	4.93	6.28	13.55	1.33
35		EB30xEB37	2R	Y	2	83.66	4.71	6.16	14.15	1.14
36		EB27xEB30/EB23xEB24	4R	Y	2	82.69	5.73	5.14	14.05	1.18
37		M53xM59/EB13	2S/R	Y	4	82.58	6.46	4.48	16.45	1.15
38		EB24xEB33/EB28xEB37	4R	Y	4	81.74	5.83	4.93	17.35	1.18
39		EB23xEB27	2R	Y	1	78.01	4.53	5.62	14.25	1.04
40		EB14xEB13	2R	W	2	58.12	4.56	3.14	15.50	1.02
41		EB23xEB30/EB27xEB30	4R	Y	2	53.92	2.63	4.33	14.50	0.91
42		EB23xEB37	2R	Y	1	48.30	2.20	4.02	14.35	0.95
		Mean of all treatments				100.00	6.97	6.18	16.17	1.30
		* Denotes Commercial Hybrids		C.V.		= 15.18	16.74			
				L.S.D. (5%)		= 2.22	2.83			

1. Based on mean relative yield

2. 4S = 4 Susceptible inbreds
RxS = 1 Resistant x 1 Susceptible inbred
2S/R = 2 Susceptible inbreds x 1 resistant

3. Y = Yellow
W = White

4. Relative to average yield for all hybrids

5. No. of ears/plant

(ii) Results and discussion

(a) Yield

No matter how resistant a hybrid is, if it does not give a competitive yield, it has no chance in the commercial market. The commercial hybrids were generally at the top of the yield rankings as this trial was not infested with borer larvae. PNR 394 (31st) is an early maturing hybrid (see data under grain moisture) in comparison to the others, and generally the quick hybrids do not have the yield potential of the long season hybrids. PNR 542 (24th) is a hybrid no longer in production, and has been superceded by other hybrids in the list. PNR 6334 (12th) is another quick maturing hybrid, expected to rank lower than the other long season hybrids. Single cross hybrids were included in the yield trial to observe the level of resistance and yield potential of such hybrids in comparison to the other hybrids. Single cross hybrids are not frequently marketed in South Africa because the yield of inbred females in production lands is much lower than yields of single cross females and production costs are high. The first three way hybrid that shows any acceptable resistance is at rank 6, where a susceptible female single cross (K10 x M24) had fairly good resistance conferred by EB37 in the final hybrid. However a resistance rating of 3 does not offer enough protection to the farmer from stalk borer damage.

At rank 9, a single cross hybrid EB62 x K10 rated 2 for stalk borer damage, and at ranks 12 and 14 two three way hybrids also rated 2. However by rank 12, the yields are already 36% below the top ranked commercial hybrid. This yield potential is obviously not a commercial proposition. The first double cross hybrid that shows any resistance is at rank 36, also obviously of no commercial use. The most resistant hybrids (rated 1) were several single crosses (ranks 22, 26, 33, 39 and 42).

It is also important that any hybrid should be as stable over locations as possible. Stability of yield under different environmental conditions is essential if a hybrid is to be commercialised successfully. Only two locations were utilized in this trial, and both unfortunately had similar yield potentials.

Once hybrids have been selected for good resistance, it is essential that they be tested in areas showing a wide range of climatic conditions, from high potential areas to stressed areas.

(b) Resistance

The commercial hybrids (rated in the observation plots in Greytown) were susceptible as expected (All rated 5). The mean resistance ratings of the other groups were 3.25 for the double crosses (range 2-4), 2.87 for the three-way crosses (range 2-4), and 2.05 for the single crosses (range 1-4). It is unfortunate that the most resistant ratings occurred in the least commercially acceptable hybrids (single crosses). However, the range of ratings for all groups indicate that acceptable resistance could be found in three-way and double cross hybrids with an extensive hybrid development programme. Such a programme is under way and, it is hoped, will result in some high yielding resistant hybrids.

(c) Grain moisture

The sooner a farmer can harvest his crop, the sooner his cash flow improves. Quick dry down, as reflected by grain moisture at harvest, is a very important attribute of hybrids. The range of moistures for the commercial hybrids ranged from 18.25% for PNR 6552 to 14.45 for PNR 6334. A moisture greater than that of PNR 6552 would cause too great a delay in harvest, so a hybrid such as EB56 x K10 (rank 5) with a moisture of 18.90% would be discarded in favour of an earlier maturing hybrid such as K10 x M24/EB37 (rank 6). No matter how good the resistance a hybrid has, if it had the moisture of EB58 x M13 (rank 10; % moisture = 20.80), it would not be a commercial proposition.

In all hybrid yield trials, generally the longer the grain filling period, the wetter the grain at harvest, and the higher up in the rankings the hybrid occurs. Conversely, the quicker a hybrid matures the lower down in the rankings it occurs. An exceptionally early single cross like EB23 x EB30 (rank 26, % moisture of 13.30) may still have a role to play in the market

place, as there is a need for quick maturing hybrids.

(d) Prolificacy

The generally accepted relationship (evident in Table 7.5.1) between prolificacy and yield shows that this is an important attribute to select for. The lower the prolificacy, the more chance there is of blind plants, especially under stress conditions. This does not mean that the hybrid EB56 x K10 (rank 5, prolificacy index of 1.07) would be discarded, as many very successful hybrids are single eared. It does mean, however, that a hybrid with similar resistance, moisture and yield, but with a higher prolificacy index, would be selected in preference.

In addition to the above attributes, cognisance must be taken of standability, cob and stem disease resistance, leaf disease resistance, and sales appearance to the farmer. The development of a commercial hybrid with borer resistance is a long process, and to be accepted on the open market, it must perform as well as susceptible hybrids in the absence of stalkborer infestations. The present state of hybrid development is obviously in its infancy. Several inbreds and single cross hybrids show excellent resistance, and it is now merely a matter of getting them into the right combinations. It is evident that the first successes will probably be achieved through three way hybrids.

8. DISCUSSION AND CONCLUSIONS ON THE POTENTIAL USE OF RESISTANT MAIZE CULTIVARS IN THE MANAGEMENT OF *BUSSEOLA FUSCA*

Mention must be made of the potential of biotechnology in controlling *B. fusca*. Lately, several researchers have developed resistance in certain crops (mainly cotton, tomatoes, potatoes and tobacco) to various insects through gene transfer. Scientists of Monsanto recently announced that they have succeeded in genetically engineering cotton for improved resistance to several Lepidopterous pests (Anon, 1989a). The researchers introduced a gene from the widely occurring bacterium *Bacillus thuringiensis* (Bt) using another bacterium (*Agrobacterium tumefasciens*) as the carrier. The protein produced by *B.thuringiensis* paralyses the insect gut by breaking down the digestive system, and causes the insect's death.

In Wisconsin, researchers of Agracetus announced that they had permission from the Animal and Plant Health Inspection Services to field test cotton which they had also genetically modified to resist attack by tobacco budworm and cotton bollworm (Anon, 1989b). Similar procedures to those used by Monsanto were utilized to introduce the Bt gene into cotton using *A.tumefasciens*.

Plant Genetic Systems of Belgium has also conducted field tests on the resistance to tobacco budworm of genetically engineered tobacco (Anon, 1989c). Their scientists found that the engineered plants provided high levels of resistance against the pest.

In all these cases, no yield loss was noted, and it appears that the insertion of such genes can be done without any loss of desirable agronomic characteristics.

Biotechnology research on cereals has not progressed at the same rate as on the crops mentioned above. As discussed elsewhere, resistance breeding against the European Corn Borer (ECB) has evolved along conventional lines, with the frequency of favourable resistance alleles being built up in a population

mainly though recurrent selection. Progress in backcross selection, designed to transfer quantitatively inherited traits, has been slow. Northrup King is currently funding a project at Iowa State University using Restriction Fragment Length Polymorphism to locate and describe chromosome segments that form part of the resistance to the European Corn Borer (Ferris, 1988; Anon, 1989d). This technique ascertains the magnitude of resistance provided by these segments. It also determines how marker facilitated selection influences other economically important traits. These traits may be inherited on chromosome segments that are closely linked with ECB - related segments and, therefore, may be carried along during selection for ECB resistance. Northrup King researchers are also looking at inserting the Bt gene into maize, but have not yet had success. They claim, however, that the first transgenic hybrid could be marketed in the mid - 1990's.

Another U.S. Company which is very active in the agricultural biotechnology field, Ecogen Inc., has recently signed an agreement with Pioneer Hi-Bred International Inc., possibly the largest maize seed company in the world (no link whatever to Pioneer Seed Company in South Africa), to collaborate on the development of hybrid maize resistant to the ECB (McIntyre, 1989). Under this agreement, the Bt gene will be genetically cloned by Ecogen and supplied to Pioneer. Several such genes have already been isolated by Ecogen. Pioneer will introduce the Bt gene into maize plants through genetic engineering techniques, which will hopefully result in resistant hybrids.

With the flurry of activity on Bt, it was deemed essential to evaluate the effect of commercial preparations of *B.thuringiensis* on *B. fusca*. The trial procedures etc. will not be reported here, but the results were totally negative - larvae of *B. fusca* feeding on leaves sprayed with various concentrations of these preparations showed no ill effects at all. If maize genotypes with the Bt gene become available, they will be screened for resistance to *B. fusca*, but it does not look a promising avenue

of research in the near future. For the forseeable future, the development of resistance to *B. fusca* will be conducted along conventional plant breeding lines as discussed above.

Several salient points have emerged in the study on the interaction between maize and *B. fusca*. The most important discovery is that it is possible to develop maize germplasm which is resistant to *B. fusca*. Through mainly recurrent recombination techniques, the small and disparate amounts of resistance which were initially identified in diverse sources of germplasm were combined in enhanced quantities in several inbreds and populations. No major dominant genes were identified, and the successful development of resistant hybrids will depend very much on combining inbreds which show specific combining ability with each other for traits such as resistance, yield, early maturity and reasonable resistance to cob, stem and leaf diseases.

The second important facet investigated was the multiple interaction of factors such as the stage of plant growth at the time of larval infestation, level of resistance in leaf tissue, numbers and biomass of larvae entering the stem, stage of plant growth when stem damage occurs, level of resistance in stem tissue, maturity of the plant under attack, and inherent yield potential of the plant. As was shown in Fig.19., an infestation of *B. fusca* in two fields of the same hybrid, but at different stages of plant growth, can result in two very different reactions. Severe yield loss can occur in plants showing a very resistant leaf reaction.

Through selection for resistance under continuous artificial infestation, it has been possible to develop inbreds and populations that show good resistance to *B. fusca* in leaf and stem tissue. If sufficient of these inbreds are tested in combination with each other, it is feasible that resistant hybrids will be developed. However, as pointed out in 7.5., the development of resistant hybrids that are commercially competitive is not an easy task. The HPR programme on *B. fusca*

is now at the stage of testing many experimental hybrids that have been made up with various combinations of resistant and susceptible inbreds. In addition to resistance, it is hoped that some may show all the important agronomic attributes required for successful commercialisation.

The over-riding questions that must be asked are : If a borer-resistant hybrid that is commercially fairly competitive is now available, does it have a place in the market place, and what benefits will accrue to the farmers who plant the hybrid in preference to other susceptible hybrids? Will the planting of such hybrids result in an overall reduction in population numbers of *B. fusca*? Will the use of resistant hybrids result in resistance to the plants developing in populations of *B. fusca*?

The third question is the easiest to answer. At present in the South African maize market, there are 5 major seed companies marketing well over 40 hybrids, although about 3 to 5 of these hybrids have over 75% of the total market. A speciality borer resistant hybrid would be distributed by only one seed company that would have sole access to the resistant germplasm. It would be planted in widely dispersed localities, probably in small acreages. As the resistance developed to *B. fusca* is polygenic, it would be very unlikely for horizontal resistance to break down in the first instance, and secondly for the entire South African stalk borer population to be exposed to these resistance genes. Continual intermating would occur between borer populations exposed to the resistance and populations arising from susceptible hybrids. The fear of *B. fusca* overcoming the resistance would therefore be totally unfounded.

As to the second question, concerning the effect of resistant hybrids on reducing *B. fusca* populations, it is unlikely that the planting of resistant hybrids would have the slightest effect on population dynamics of *B. fusca*. For well over 50 years, maize farmers have been combatting *B. fusca* with all means at their disposal, and many researchers agree that *B. fusca* is still the

most common and widespread pest of maize. There are always farmers who do not control the borer efficiently, and whose crops therefore serve as reservoirs for future infestations. Moths can fly over large distances, and reinfestation always occurs on lands that had excellent borer control in previous seasons. The insect is just too mobile for any attractive crop to escape infestation. Populations would always be in a constant state of flux and movement would occur at random over the entire maize growing area.

The first question is actually the "raison d'etre" of the HPR programme on *B. fusca* . The Maize Stalk borer is currently controlled predominantly by chemical means. Chemicals cost money, and farmers are continually reviewing input costs and ways of reducing expenditure. Whether a farmer will decide to plant a borer-resistant hybrid depends on one single question : "does the hybrid yield as well as other commercial hybrids under conditions of nil, or low, infestation?" If that answer is affirmative, then a farmer will most definitely plant a borer-resistant hybrid in preference to a susceptible one. But, as discussed in 7.5., it appears that the development of an agronomically acceptable borer-resistant hybrid is a long way off. If the answer is negative, then other benefits must accrue for the hybrid to see the commercial light of day. A lower yield potential must be balanced against a saving in costs and time through not having to control any infestations. Overman (1986) concluded that Fall Armyworm resistance must be demonstrated in the field, and resistant hybrids must be competitive with other commercial hybrids in the absence of the pest, as well as demonstrate a significant yield advantage under infestation.

At the present time, control methods for stalk borer in maize include the placement of granular systemic insecticides in the planting furrow, spraying of liquid chemicals by tractor or by air, and the placement of granules down the funnel by tractor or by hand. Costs range from *ca.* R140/ha if a systemic carbamate is applied at planting in the furrow, with liquid sprays costing *ca.*

R14 - R18/ha, down to *ca.* R10/ha for hand applied granules. Application costs would have to be included in the overall costing, and these would range from *ca.* R20/ha for aerial spraying, *ca.* R5/Ha for tractor spraying, to *ca.* R1/ha for hand applied granules. It is therefor difficult to quantify costs as so many chemicals and so many methods are used by different farmers. These would range from R145/ha for a farmer applying a systemic chemical at planting to R22/ha for two carefully timed applications of hand applied granules.

In addition, the yield potential of the crop on that particular land would have to be included in the decision to plant a lower yielding resistant hybrid. The economic threshold for *B. fusca* is still being investigated by J.v.Rensburg[8] (pers.comm.), and it is obvious that the level of infestation will have an important bearing on whether or not a farmer will apply corrective control measures.

The grain price is obviously another factor that must be considered in such a calculation. At the present time, the grain price is R240/Tonne for yellow maize and R235/Tonne for white maize. A 10% reduction in yield potential is obviously worth more to a farmer expecting 10Tonnes/ha of grain (a R240 loss) than to a farmer expecting a 2Tonne/ha crop (a R48 loss). The former would obviously rather plant a higher yielding susceptible hybrid, and then control any infestation that may develop.

The latter farmer could get by with two applications of granules costing R22/ha, so it would be advantageous to plant a susceptible but high yielding hybrid.

If the resistant hybrid is only 5% lower yielding than other commercial hybrids, the farmer in the high potential area would only lose grain to the value of R120, but would save R145 in control costs, giving a nett saving of R25/ha. The farmer in the

---

[8] J.van Rensburg, Grain Crops Research Institute, Box X804, Potchefstroom, South Africa.

low potential area would lose R24 in reduced yield, but could have spent only R22/ha in control costs. If a third treatment was required, he would obviously be better off planting the resistant hybrid.

In addition, there are many peasant farmers who do not have access to sufficient finance to apply the required inputs such as fertilizers, herbicides and insecticides. Experience at field days of the enthusiasm of such farmers for insect resistant hybrids would indicate a suitable market for lower yielding, but resistant, hybrids that did not require the farmer to purchase insecticides.

All these examples have assumed that the borer-resistant hybrid effectively controlled all larvae, or that any surviving larvae did not cause any economic damage.

It is obvious that many diverse factors have to be taken into account when assessing the worth of planting a resistant hybrid that may be slightly lower yielding than a susceptible, but higher yielding, hybrid. The one factor that has not been considered is the farmer himself. Many farmers plant hybrids that are not the highest yielding cultivars purely because of such diverse factors as what hybrid his neighbour plants, how well he gets on with the seed company representatives, and the general appearance in the field of the hybrid. It is evident that the farmer is the most strategic strand in the whole web of hybrid selection. The decision to select one hybrid over another is often an uncalculated one, based on the persuasiveness and credibility of the representative. It is quite possible that this is the only way that farmers will be convinced to plant a resistant but lower yielding hybrid. The scientific modelling of all the factors described above which affect a farmer's maize crop could be no match for a persuasive representative, and in the end that could be the deciding factor in the commercialisation of a borer-resistant hybrid.

CHAPTER 9

SUMMARY

1.1. The various terms and types of resistance used in HPR research are explained and discussed. The essential requirements in any investigation into plant resistance are dealt with, and examples are given of the genetics of resistance to several borers. Initial studies in 1930 on the European Corn Borer showed the resistance to be due to a single recessive gene. In 1948, it was suggested that borer resistance was additive. It was theorised in 1953 that 2 genes were involved, and further research concluded that 3 genes were involved. It was evident from these studies that the different genotypes under study had a strong bearing on the conclusions regarding the genetics of resistance. This is borne out in the various studies contained in this thesis. Research on the Corn Earworm and Fall Armyworm also indicated that resistance was quantitatively inherited, and this is also the conclusion on the inheritance of resistance to *B. fusca*.

1.2. Early research on resistance to borers is reviewed with discussions on infestation methods, laboratory rearing, and damage rating methods. Due to the similarity in behaviour and feeding sites of the major borer pests of maize, similar methods are utilized in HPR research on many different borers.

1.3. The various requirements for a resistance programme to borers are discussed.

1.4. An in depth review is given of research carried out in maize on *B.fusca*. Topics covered include the history and geographical occurrence of the insect, its general biology, its economic importance, and history of resistance breeding.

B. fusca originated in Africa as a borer of sorghums in tropical and sub-tropical areas. It is currently under study in several Africa countries, and is considered the most serious pest of maize in all major maize growing areas of Africa. Its biology has been intensively studied since 1920, and is still the object of continuing studies. The general life cycle and its interaction with the maize crop is discussed.

Studies on the economic importance of *B.fusca* have centred predominantly around chemical control, and the determination of an economic threshold.

Evaluation of resistance to *B.fusca* occurred initially in 1943 on naturally infested plants. Different maize varieties showed different levels of damage. However these differences were ascribed to differences in growth rates and the researchers concluded that no resistance occurred in the varieties studied. At various times from 1953 to 1984, attempts to breed for resistance were carried out in South Africa, when artificial infestation was utilized. Variations in damage were ascribed to resistance in the different plant genotypes. Elsewhere in Africa, research was also carried out on resistance in sorghum to various borers.

1.5. Several aspects of the general methodology of breeding for resistance to *B. fusca* are discussed. The collection of overwintering larvae from the field in winter is described. The termination of winter diapause results eventually in first instar larvae being utilised in spring and summer in field infestations of breeding material.

About 20 first instar larvae are applied to the whorls of plants approximately 30 - 40 cm tall. Damage is assessed 20 - 25 days later by rating the extent of leaf feeding. It was concluded that field selection for resistance should rely

on leaf damage recorded after about 24 days feeding as well as visual assessment of plant height and yield at harvest.

1.6. A large section of the thesis is devoted to the effect of plant resistance on *B. fusca*. Initial studies on moth avoidance of certain maize genotypes were abandoned for many reasons discussed fully in the text. Investigations into the effects on larvae of resistance factors in leaf tissue revealed that :

(i) Application of increasing numbers of larvae up to *ca.* 20 larvae/plant resulted in greater percentage recoveries. More than 30 larvae/plant was wasteful, and it was concluded that the most economical application was between 16 - 22 larvae/plant for routine screening of maize germplasm.

(ii) There were clearly defined differences in leaf damage evident between different maize genotypes. Heritability of this resistance was demonstrated. The resistance was presumed to be an additive mechanism that reduced insect feeding, indicating antibiosis.

(iii) Larval survival and development in different maize genotypes that were subjected to various infestation levels were investigated. There were significant differences between the number of larvae recovered from each cultivar, and from different infestation levels.

Two mechanisms affecting larval numbers were present in different cultivars. One was antibiosis, resulting in larval death, and the other was repellence, resulting in larvae migrating out of the plant. Most of the migration occurred within the first seven days' feeding. Both mechanisms resulted in fewer larvae feeding in the plants.

(iv) There were also highly significant differences evident between the mean larval mass of larvae removed from each cultivar.

(v) It was concluded that three separate mechanisms are involved in resistance to *B. fusca*. Two affect numbers of larvae and the other affects larval growth and mass gain. The presence of any of these mechanisms results in a reduced larval biomass and hence reduced damage. These mechanisms were found to be heritable traits.

(vi) Two genotypes under investigation showed a large larval biomass but very low leaf damage. It was surmised that the nutritional status of these two cultivars was high, allowing larvae to consume the same amount of leaf tissue as larvae feeding in other resistant cultivars, but to gain mass more quickly.

(vii) There were no differences between larval survival in either hybrids or inbreds. The larger hybrid plants did not necessarily lead to larger or more numerous larvae.

1.7. Differences in levels of resistance affecting larval mass gain were also determined for various parts in the tassel tissue of different maize genotypes. There was no similarity in the levels of resistance recorded in a single cultivar between the various parts of the tassel. Some inbreds showed more resistance in the tassel stem, while others had more resistant glumes. The peduncles of all inbreds were more susceptible than the stems and glumes. There was no antibiotic effect on larval numbers. Large differences in tassel tissue resistance were evident between cultivars. As larvae feed for only a short while on tassel tissue,this resistance was not deemed to be important.

1.8. The other major portion of the thesis deals with the effect of *B. fusca* on the plant.

(i) The most obvious, and easily rated, type of damage is leaf damage. When different genotypes were infested, significant differences occurred in leaf damage. Various infestation levels were investigated, and it was confirmed that an application of *ca.* 20 larvae/plant was the most efficient infestation level that produced sufficiently diverse damage responses and utilised the most economical number of larvae.

(ii) The longer larvae fed, the more severe the damage. It was concluded that, for maximal expression of leaf damage, it is obviously beneficial to delay leaf damage ratings to anytime after 21 days feeding, but prior to tassel emergence.

(iii) Resistance in leaf tissue did not necessarily mean that resistance occurred in the stem of that genotype. Varying levels of stem damage occurred in different genotypes. Some cultivars had resistance mechanisms present in both leaves and stems, some had only one resistance mechanism in either part, and some were totally susceptible.

(iv) Yield was predominantly affected by severe stem damage, which also resulted in stunting of infested plants. Yield loss was more pronounced in longer season hybrids than in quick maturing hybrids.

1.9. A flowchart is presented which shows the intricate interaction between plant and insect. It illustrates the complexities of all factors involved in this interaction, especially the age of plant at infestation, the level of resistance in both stem and leaf tissue, and the maturity of the hybrid.

1.10. Methodologies utilised in the development of inbreds, populations and hybrids are discussed. Since the inception

of the research programme, progress was achieved in increasing the level of resistance in inbreds. The correct placement of the resistant and susceptible inbreds in borer resistant hybrids was shown to be critical if the resultant hybrid was to show good resistance. What complicates the matter further is that one has to take cognisance of other agronomic attributes in the making of hybrids.

1.11. It was concluded that borer resistant hybrids do have a place in the commercial market. However their performance under conditions of nil or low infestation must be similar to that of other susceptible hybrids because control measures for *B.fusca* are not excessively expensive. There would appear to be a greater demand (albeit a smaller market) for such hybrids by peasant farmers, who find prohibitive even the small cost of chemical control.

CHAPTER 10

REFERENCES

ANNEKE, D.P. & MORAN, V.C. (1982). *Insects and Mites of Cultivated Plants in South Africa*. Pretoria : Butterworths.

ANON. (1967). Plant Protection Branch Annual Report 1967 - 1978. Research & Specialist Services, Department of Agriculture, Rhodesia.

ANON. (1969). Chemistry and Soil Science Branch Annual Report 1969 - 1970. Research & Specialist Services, Department of Agriculture, Rhodesia.

ANON. (1970). Chemistry and Soil Science Branch Annual Report 1970 - 1971. Research & Specialist Services, Department of Agriculture, Rhodesia.

ANON. (1973). Plant Protection Research Institute Annual Report 1973 - 1974. Research & Specialist Services, Department of Agriculture, Rhodesia.

ANON. (1974). Plant Protection Research Institute Annual Report 1974 - 1975. Research & Specialist Services, Department of Agriculture, Rhodesia.

ANON. (1989a). Genetically altered cotton resists certain insects. In *Seed Trade News* May 3 : 18.

ANON. (1989b). First field testing of genetically engineered cotton plants underway. In *Seed Trade News* June 7 : 13.

ANON. (1989c). Belgian biotech firm expands field trials. In *Seed World* May : 33 - 35.

ANON. (1989d). Iowa focuses on European Corn Borer. In *Seed Trade News* June 7 : 6.

ATKINSON, P.R. (1978). Mass rearing and artificial infestation methods for *Eldana saccharina* Walker. *Proceedings of the South African Sugar Technologists' Association* June 1978 : 1 - 3.

BARROW, M.R. (1985). The effect of different maize genotypes on the maize stalk-borer, *Busseola fusca* (Fuller)(Lepidoptera: Noctuidae), feeding in whorl tissue. *Journal of the entomological Society of South Africa* **48(1)** : 113 - 119.

BARROW, M.R. (1987). The effect of first generation maize stalkborer, *Busseola fusca* (Fuller) (Lepidoptera:Noctuidae), on yield of different maize genotypes. *Journal of the entomological Society of South Africa* **50(2)** : 291 - 298.

BARROW, M.R. (1989). Screening and breeding for resistance to *Busseola fusca*. In *CIMMYT. 1989. Toward insect resistant maize for the third world : Proceedings of the International Symposium on Methodologies for Developing Host Plant Resistance to Maize Insects*. Mexico, D.F. : CIMMYT.

BECK, S.D. & SCHOONHOVEN, L.M. (1980). Insect behaviour and plant resistance. In *Breeding Plants Resistant to Insects*, ed. Maxwell F.G. & Jennings, P.R. New York : John Wiley & Sons.

BEMILLER, J.N. & PAPPELIS, J.A. (1965). 2,4 - dihydroxy-7-methoxy-1,4-benzoxazin-3-one glucoside in Corn. 1. Relation of water soluble 1-butanol soluble fraction content of pith cores and stalk rot resistance. *Phytopathology* **55** : 1237 - 1240.

BLAIR, B.W. & READ, J.S. (1969). A preliminary report on the sex pheromone of the maize stalk-borer *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae). *Rhodesian Journal of agricultural Research* 7 : 55 - 59.

BLAIR, B.W. (1971). Recent research on the maize stalk-borer *Busseola fusca* (Fuller) : Influence on control methods. *Rhodesian agricultural Journal* **68** : 111 - 112.

BRINDLEY, T.A. & DICKE, F.F. (1963). Significant developments in European Corn Borer research. *Annual Review of Entomology* **8** : 155 - 176.

CIMMYT. (1987). 1986 CIMMYT world maize facts and trends : The economics of commercial maize seed production in developing countries. Mexico, D.F. : CIMMYT.

DAHMS, R.G. (1972). Techniques in the evaluation and development of host-plant resistance. *Journal of environmental Quality* **1** : 254 - 259.

DAVIS, F.M. & WILLIAMS, W.P. (1979). More efficient procedures for screening corn genotypes for resistance to second-brood Southwestern Corn Borer. *Journal of economic Entomology* **72(5)** : 801 - 803.

DAVIS, F.M. (1980). A larval Dispenser-Capper machine for mass rearing the Southwestern Corn Borer. *Journal of economic Entomology* **73(5)** : 692 - 693.

DAVIS, F.W. & WILLIAMS, W.P. (1980). Southwestern Corn Borer : Comparison of techniques for infesting corn for plant resistance studies. *Journal of economic Entomology* **73(5)** : 704 - 706.

DAVIS, F.M. & WILLIAMS, W.P. (1983). Second-generation Southwestern Corn Borer (Lepidoptera:Pyralidae): Ear and stalk damage to susceptible and resistant maize. *Journal of economic Entomology* **76(3)** : 507 - 509.

DAVIS, F.M. & WILLIAMS, W.P. (1986). Survival, growth, and development of Southwestern Corn Borer (Lepidoptera:Pyralidae) on resistant and susceptible maize hybrids. *Journal of economic Entomology* **79(3)** : 847 - 850.

DICKE, F.F. (1954). Breeding for resistance to European Corn Borer. *Proceedings of the Ninth Annual Hybrid Corn Industry-research Conference* : 44 - 53.

DRINKWATER, T.W., WALTERS, M.C. & VAN RENSBURG, J.B.J. (1979). The application of systemic insecticides to the soil for the control of the maize stalkborer, *Busseola fusca* (Fuller) (Lep : Noctuidae) and of *Cicadulina mbila* (Naudé) (Hem : Cicadellidae), the vector of maize streak virus. *Phytophylactica* **11** : 5 - 12.

DU PLESSIS, C.& LEA, H.A.P. (1943). The Maize Stalk-borer, *Calamistis fusca*. *South African Department of Agriculture Bulletin* **238** : 1 - 51.

ELLINGER, T. & CHORINE, V. (1930). Report on a corn variety test under corn borer conditions. International Livestock Experiments (Chicago). *International Corn Borer Investigations and Scientific Reports* 3 : 99 - 100.

ELLINGER, T. & CHORINE, V. (1931). Comparison de la resistance du mais sud-africain et du mais American a l'infection par la pyrale du mais (*Pyrausta nubilalis* Hub.). *Annals de l'Institute Pasteur* **46(4)** : 480-481.

ELNAGHY, M.A. & LINKO, P. (1962). The role of 4-0-glucosy1-2, 4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one in resistance of wheat to stem rust. *Physiology Plantarum* **15** : 764 - 771.

FARIS, M.A., DE A.LIRA, M. & VEIGA, A.F.S.L. (1976). Sorghum midge evaluation of the Texas Agricultural Experiment Station International Insect Nursery, Serra Talhada Research Station, Pe., Brazil, 1975. *Sorghum Newsletter* **19** : 4.

FERRIS, R.(1988). Research advances on European Corn Borer Resistance. In *Seed World* December : 36 - 37.

FOSTER, J.E. & ORTMANN E.E. (1988). Annual plant resistance to insects newsletter. **Volume 14.**

Ficht, G.A. (1936). The European Corn Borer in Indiana. *Purdue University Agricultural Experimental Station Bulletin* **No. 406** : 1 - 24.

FOURIE, A.P. (1984). Variation in resistance of maize inbred lines to *Busseola fusca* and *Chilo partellus*. *Proceedings of the 6th South African Maize Breeding Symposium* : 56 - 57.

GALLUN, R.L. & KHUSH, G.S. (1980). Genetic factors affecting expression and stability of resistance. In *Breeding Plants Resistant to Insects*, ed. Maxwell F.G. & Jennings, P.R. New York : John Wiley & Sons.

GEBRE-AMLAK, A. (1988). Survival of maize stalk borer, *Busseola fusca* (Fuller), in crop residues in Ethiopia. *Crop Protection* 7 : 183 - 185.

GHIDIU, G.M., BERRY, E.C. & DuBOSE, P. (1979). Relationship between entrance holes and stalk cavities caused by 1st-, and 2nd- generation European Corn Borer in field corn. *Journal of economic Entomology* **72(1)** : 85 - 86.

GRIER, S.L. & DAVIS, D.W. (1980). Infestation procedures and heritability of characters used to estimate ear damage caused by second-brood European Corn Borer (*Ostrinia nubilalis*, Hubner) on corn. *Journal of the American Society for horicultural Science* **105(1)** : 3 - 8.

GUTHRIE, W.D., DICKE, F.F. & NEISWANDER, C.R. (1960). Leaf and sheath feeding resistance to the European Corn Borer in eight inbred lines of dent corn. *Research Bulletin* **860**. Ohio Agricultural Experiment Station, Wooster, Ohio.

GUTHRIE, W.D., HUGGANS, J.L. & CHATTERJI, S.M. (1970). Sheath and collar feeding resistance to the second-brood European Corn Borer in six inbred lines of dent corn. *Iowa State Journal of Science* **44(3)** : 297 - 311.

GUTHRIE, W.D., RUSSELL, W.A. & JENNINGS, C.W. (1971). Resistance of maize to second-brood European Corn Borers. *Annual Corn and Sorghum Research Conference Proceedings* **26** : 165 - 179.

GUTHRIE, W.D., RUSSELL, W.A., REED, G.L., HALLAUER, A.R. & COX, D.F. (1978). Methods of evaluating maize for sheath-collar feeding resistance to the European Corn Borer. *Maydica* **23** : 45 - 53.

GUTHRIE, W.D., ONUKOGU,F.A., AWADALLAH, W.H. & ROBBINS, J.C. (1980). European Corn Borer: Evaluation of resistance in husk-silk tissue of inbred lines of corn. *Journal of economic Entomology* **73(1)** : 178 - 180.

GUTHRIE, W.D., JARVIS, J.L.,REED, G.L. & LODHOLZ, M.L. (1982). Plant damage and survival of European Corn Borer cultures reared for 16 generations on maize plants and for 120 generations on a Meridic diet (one generation per year on resistant or susceptible maize plants, eight generations per year on the diet). *Journal of economic Entomology* **75(1)** : 134 - 136.

GUTHRIE, W.D., JARVIS, J.L. & ROBBINS, J.C. (1984). Damage from infesting maize plants with European Corn Borer egg masses and larvae. *Journal of agricultural Entomology* **1(1)** : 5 - 16.

GUTHRIE, W.D., RUSSELL, W.A., JARVIS, J.L. & ROBBINS, J.C. (1985). Performance of maize inbred line B86 in hybrid combinations: Resistance to first- and second-generation European Corn Borers (Lepidoptera:Pyralidae). *Journal of economic Entomology* **78(1)** : 93 - 95.

HALL, P.K., PARROTT W.L., JENNINGS, J.N. & MCCARTY J.C. (1980). Use of tobacco budworm eggs and larvae for establishing field infestations on cotton. *Journal of economic Entomology* **73** : 393 - 395.

HALL, D.R., BEEVOR, P.S., CORK, A., LESTER, R., NESBITT, BRENDA F., NYIRENDA, G.K.C., NOTA PHIRI, D.D., BLAIR, B.W., & TANNOCK, J. (1981). The female sex pheromone of the maize stalk-borer *Busseola fusca* (Fuller)(Lepidoptera: Noctuidae): Identification and initial field trials. *Zimbabwe Journal of agricultural Research* **19** : 111 - 123.

HAMILTON, R.H. (1964). A corn mutant deficient in 2,4 - dihydroxy-7-methoxy-1,4-benzoxazin-3-one with an altered tolerance of atrazine. *Weeds* **12** : 27 - 30.

HARRIS, K.M. (1962). Lepidopterous stem borers of cereals in Nigeria. *Bulletin of entomological Research* **53** : 139 - 171.

HARRIS, K.M. (1964). Annual variations of dry-season populations of larvae of *Busseola fusca* (Fuller) in Northern Nigeria. *Bulletin of entomological Research* **54** : 643 - 647.

HASE, A. (1929). Report on corn borer experiments 1927 - 28. International Livestock Experiments (Chicago). *International Corn Borer Investigations and Scientific Reports* **2** : 77 - 84.

HILL, R.J. (1973). A review of *Busseola fusca*, with suggestions on future lines of investigation. Salisbury: Ministry of Agriculture.

HORBER, E.(1980). Types and classification of resistance. In *Breeding Plants Resistant to Insects*, ed. Maxwell F.G. & Jennings, P.R. New York : John Wiley & Sons.

INGRAM, W.R. (1958). The Lepidopterous stalk borers associated with Gramineae in Uganda. *Bulletin of entomological Research* **49** : 367 - 383.

JACK, R.W. (1917). The maize stalk borer : General account of life history and control measures. *Department of Agriculture of Rhodesia Bulletin* **276**.

JARVIS, J.L.,GUTHRIE, W.D.& ROBBINS, J.C. (1986). Yield losses from second-generation European Corn Borers (Lepidoptera:Pyralidae) in long-season maize hybrids planted early compared with short-season hybrids planted late. *Journal of economic Entomology* **79(1)** : 243 - 246.

JEPSON, W.F. (1954). A critical review of the world literature on the lepidopterous stalkborers of the tropical graminaceous crops. *Commonwealth Institute of Entomology, London.*

JUGENHEIMER, R.W. (1976). *Corn improvement, seed production, and uses* New York : John Wiley and Sons Inc.

KAUFMANN, T. (1983). Observations on the host plant adaptation of *Busseola fusca* (Lepidoptera:Noctuidae) in Nigeria. *Proceedings of the entomological Society of Washington* **85(2)** : 321 - 326.

KLENKE, J.R., RUSSELL, W.A. & GUTHRIE, W.D. (1986). Grain yield reduction caused by second generation European Corn Borer in BS9 corn synthetic. *Crop Science* **26** : 859 - 863.

KLUN, J.A. & BRINDLEY, T.A. (1966). Role of 6-Methoxybenzoxazolinone in inbred resistance of host plant (maize) to first-brood larvae of European Corn Borer. *Journal of economic Entomology* **59(3)** : 711 - 718.

KLUN, J.A. & ROBINSON, J.F. (1969). Concentration of two 1,4 - benzoxazinones in dent corn at various stages of development of the plant and its relation to resistance of the host plant to the European Corn Borer. *Journal of economic Entomology* **62** : 214-220.

KOGAN, M. & ORTMAN, E.E. (1978), Antixenosis - a new term proposed to replace Painter's "non-preference" modality of resistance. *ESA Bulletin* **24**.

KUHN, H.C. (1978). Selection for resistance against the maize stalk borer (*Busseola fusca*). *Proceedings of the 3rd South African Maize Breeding Symposium* : 84 - 86.

LUGINBILL, P.Jr. (1969). Developing resistant plants - the ideal method of controlling insects. *U.S. Department of Agriculture, A.R.S. Production and Research Report* **111** : 14 pp.

LYNCH, R.E., ROBINSON,J.F. & BERRY, E.C. (1980). European Corn Borer: Yield losses and damage resulting from a simulated natural infestation. *Journal of economic Entomology* **73(1)** : 141 - 144.

MALLY, C.W. (1920). The Maize Stalk Borer, *Busseola fusca*, Fuller. *South African Department of Agriculture*. Bulletin 3.

MARSTON, A.R. (1930). Breeding corn for resistance to the European Corn Borer. *American Society of Agronomy Journal* **22** : 986 - 992.

MARSTON, A.R. & DIBBLE, C.B. (1930). Investigations of corn borer control at Monroe, Michigan. *Michigan State University Agricultural Experimental Station Special Bulletin* **No. 204**, 47pp.

MARSTON, A.R. (1933). Recent progress in breeding borer-resistant corn. *Michigan Agricultural Experimental Station Quarterly Bulletin* **15(4)** : 264 - 268.

MATTHEE, J.J., BEDFORD, E.C.G., BROWN, H.D., LOMBARD, J., MEYER, M.P.K., VAN RENSBURG, N.J. & WALTERS, S.S. (1971). Insect pests of grain and oil seed crops. Paper presented at the National Entomological Symposium, Pretoria. Sept. 1971.

MCINTYRE, J.L. (1989). Ecogen and Pioneer Hi-bred to collaborate on insect-resistant corn research. *News release*, Ecogen, Langhorne Pennsylvania.

MAXWELL, F.G. (1972). Host plant resistance to insects - nutritional and pest management relationships. In *Insect and Mite Nutrition*, Amsterdam : North Holland.

MAXWELL F.G & JENNINGS, P.R. (1980) *Breeding Plants Resistant to Insects*. New York : John Wiley & Sons.

MIHM, J.A. (1983). Efficient mass rearing and infestation techniques to screen for host plant resistance to maize stem borers, *Diatraea* sp. Mexico, D.F : CIMMYT.

MIHM, J.A. (1985). Breeding for host plant resistance to maize stem-borers. *Insect Science and its Application* **6(3)** : 369 - 377.

MLAMBO, S.S. (1983). The status of cereal stem-borer and legume pod-borer research in Zimbabwe. *Insect Science and its Application* **4(1)** : 221 - 222.

MOHYUDDIN, A.I. & GREATHEAD, D.J. (1970). An annotated list of the parasitoids of graminaceous stem borers in East Africa, with a discussion of their potential in biological control. *Entomophaga* **15** : 241 - 274.

NORIS, D.M. & KOGAN M (1980). Biochemical and morphological bases of resistance. In *Breeding Plants Resistant to Insects*, ed. Maxwell. F.G. & Jennings, P.R. New York : John Wiley & Sons.

NYHUS, K.A., RUSSELL, W.A. & GUTHRIE, W.D. (1988). Response of two maize synthetics to recurrent selection for resistance to first generation European Corn Borer (Lepidoptera : Pyralidae) and *Diplodia* Stalk rot. *Journal of economic Entomology* **81(6)** : 1792 - 1798.

ORTEGA, A., VASCAL, S.k., MIHM, J. & HERSHEY C. (1980). Breeding for insect resistance in maize. In *Breeding Plants Resistant to Insects*, ed. Maxwell F.G. & Jennings, P.R. New York : John Wiley & Sons.

ORTEGA, A C. (1987). *Insect Pests of Maize : A Guide for Field Identification*, Mexico, D.F. : CIMMYT.

OVERMAN, J.L. (1986). Resistance in corn (Zea mays) to the Fall Armyworm (*Spodoptera frugiperda*) : The impact of public research on commercial seed companies. *The Florida Entomologist* **69(3)** : 537 - 541.

OVERMAN, J.L. (1989). A Maize breeding program for development of hybrids with resistance to multiple species of leaf-feeding and stalk-boring Lepidoptera. *In CIMMYT. 1989. Toward Insect resistant maize for the third world: Proceedings of the Internaional Symposium on Methodologies for Developing Host Plant Resistance to Maize Insects. Mexico, D.F.: CIMMYT.*

PAINTER, R.H. (1951). *Insect Resistance in Crop Plants*. New York : The MacMillan Company.

PATCH, L.H. (1942). Height of corn as a factor in egg laying by the European corn Borer moth in the one generation area. *Journal of Agronomy Research* **64** : 503 - 515.

PATHAK, M.D. (1975). Utilization of insect-plant interactions in pest control. In *Insects, Science and Society*. New York : Academic Press.

PENNY, L.H. & DICKE, F.F. (1956). Inheritance of resistance to leaf feeding of the European Corn Borer. *Agronomy Journal* **48** : 200 - 203.

PIMENTAL, D. (1969). Animal populations, plant host resistance and environmental control. *Proceedings of the Tall Timbers Conference on Ecology, Animal Control and Habitat Management* **1** : 19 - 28.

ROUBOUD, E. (1928). Biological researches on *Pyrausta nubilalis* H6. International Livestock Experiments (Chicago). *International Corn Borer Investigations and Scientific Reports* **1920 - 1927** : 1 - 40.

REVINGTON, J., VAN RENSBURG,J.B.J., BURGHARDT, G. & KNAUF, W. (1984). Preliminary field trials with a pheromone based monitoring system for the maize stalkborer, *Busseola fusca* (Fuller) (Lep:Noctuidae). *Journal of the entomological Society of South Africa* **47(1)** : 107 - 113.

ROBERTSON, D.S. & WALTER, E.V. (1963). Genetic studies of earworm resistance in maize. Utilizing a series of chromosome-nine translocations. *Journal of Heredity* **54** : 267 - 272.

ROBINSON, J.F., KLUN, J.A. & BRINDLEY, T.A. (1978). European Corn Borer: A nonpreference mechanism of leaf feeding resistance and its relationship to 1,4-Benzoxazin-3-one concentration in dent corn tissue. *Journal of economic Entomology* **71(3)** : 461 - 465.

ROSE, D.J.W. (1962). Pests of maize and other cereal crops in the Rhodesias. *Bulletin of the Federation Ministry of Agriculture, Rhodesia and Nyasaland* **163** : 1 - 23.

ROSE, D.J.W. (1976). The relationship between maize pests and crop loss. *Rhodesian agricultural Journal* **73** : 139 - 140.

SCHLOSBURG, M. & BAKER, W.A. (1948). Tests of sweet corn lines for resistance to European Corn Borer larvae. *Journal of agricultural Research* **77(5)** : 137 - 156.

SCHROEDER, A.C., MASON, C.E., BLANKENSHIP, E.A.K. & REESE, J.C. (1986). Evaluation of pinto bean, wheat germ, and lima bean diets for rearing the European Corn Borer (Lepidoptera : Pyralidae). *Journal of economic Entomology* **79(4)** : 964 - 969.

SESHU REDDY, K.V. (1985). Relative susceptibility and resistance of some sorghum lines to stem-borers in Western Kenya. *Insect Science and its Application* **6(3)** : 401 - 404.

SINGH, R. (1953). Inheritance in maize of reaction to the European Corn Borer. *Indian Journal of Genetics and Plant Breeding* 13 : 18 - 47.

SMITHERS, C.N. (1960). Some recent observations on *Busseola fusca* (Fuller) (Lep., Noctuidae) in Southern Rhodesia. *Bulletin of entomological Research* **50** : 809 - 819.

SPRAGUE, G.F. (1977). Corn and corn improvement. *Monograph 18. American Society of Agronomy* : Madison, Wisconsin.

STARKS, K.J., BURTON, R.L., WILSON, R.L. & DAVIS, F.M. (1982). Southwestern Corn Borer: Influence of planting dates and times of infestation on damage to corn, pearl millet, and sorghum. *Journal of economic Entomology* **75(1)** : 57 - 60.

STRONG, D.R., LAWTON, J.H. & SOUTHWOOD, R (1984). Insects on Plants : Community Patterns and Mechanism. London : Blackwell Scientific Publications.

SWAINE, G.(1957). The maize and sorghum stalkborer, *Busseola fusca* (Fuller), in peasant agriculture in Tanganyika territory. *Bulletin of entomological Research* **48** : 711 - 722.

TAMS, W.H.T. & BOWDEN, J. (1953). A revision of the African species of Sesamia Guenee and related genera (Agrotidae-Lepidoptera). *Bulletin of Entomological Research* **43** : 645 - 679.

UMEOZOR, O.C., VAN DUYN, J.W., KENNEDY, G.G. & BRADLEY JR.,J.R. (1985). European Corn Borer (Lepidoptera:Pyralidae) damage to maize in Eastern North Carolina. *Journal of economic Entomology* **78(6)** : 1488 - 1494.

UNNITHAN, G.C. (1987). Development and reproductive biology of the maize stem-borer *Busseola fusca* Fuller (Lepid.,Noctuidae). *Journal of applied Entomology* **104(2)** : 172 - 179.

USUA, E.J. (1968a). Effect of varying populations of *Busseola fusca* larvae on the growth and yield of maize. *Journal of economic Entomology* **61(2)** : 375 - 376.

USUA, E.J. (1968b). The biology and ecology of *Busseola fusca* and *Sesamia* species in South-Western Nigeria. I.Distribution and population studies. *Journal of economic Entomology* **61(3)** : 830 - 833.

USUA, E.J. (1970a). Some notes on maize stemborers in Nigeria. *Journal of economic Entomology* **63(3)** : 776 - 778.

USUA, E.J. (1970b). Diapause in the maize stemborer. *Journal of economic Entomology* **63(5)** : 1605 - 1610.

USUA, E.J. (1974). Observations on the physiology of diapause and non-diapause larvae of *Busseola fusca* (Fuller) (Lep.,Noctuidae). *Bulletin of entomological Research* **63** : 513 - 518.

VAN DER PLANK, J.E. (1968). *Disease Resistance in Plants*. New York : Academic Press.

VAN RENSBURG, J.B.J. & WALTERS, M.C. (1978). The efficacy of systemic insecticides applied to the soil for control of *Cicadulina mbila* (Naudé) (Hem. : Cicadellidae) the vector of maize streak disease, and the maize stalk borer, *Busseola fusca* (Fuller)(Lep.: Noctuidae). *Phytophylactica* **10** : 49 - 52.

VAN RENSBURG, J.B.J., WALTERS, M.C. & GILIOMEE, J.H. (1987a). Ecology of the maize stalkborer, *Busseola fusca* (Fuller) (Lepidoptera:Noctuidae). *Bulletin of entomological Research* **77** : 255 - 269.

VAN RENSBURG, J.B.J., VAN RENSBURG, G.D.J., GILIOMEE, J.H. & WALTERS, M.C. (1987b). The influence of rainfall on the seasonal abundance and flight activity of the maize stalk borer, *Busseola fusca* in South Africa. *South African Journal of Plant and Soil* **4(4)** : 183 - 187.

VAN RENSBURG, J.B.J., VAN RENSBURG, G.D.J. & MALAN, E.M. (1980). An evaluation of synthetic pyrethroid insecticides for the control of the Maize stalk borer, *Busseola fusca* (Fuller) (Lep. : Noctuidae) on maize and grain sorghum. *Crop Production* **9** : 157 - 162.

VAN RENSBURG, J.B.J. (1981). Die plaagstatus van *Busseola fusca* (Fuller) : 'n studie van plaag - tot - gewasverhoudings. Ph.D. thesis, University of Stellenbosch.

VAN RENSBURG, J.B.J., WALTERS, M.C., & GILIOMEE, J.H. (1988a). Response of maize to levels and times of infestation by *Busseola fusca* (Fuller) (Lepidoptera:Noctuidae). *Journal of the entomological Society of South Africa* **51(2)** : 283 - 291.

VAN RENSBURG, J.B.J., WALTERS, M.C. & GILIOMEE, J.H. (1988b). Mortality in natural populations of the maize stalk borer, *Busseola fusca* (Fuller) (Lepidoptera:Noctuidae) in South Africa. *Phytophylactica* **20** : 17 - 19.

VAN RENSBURG, J.B.J.,GILIOMEE, J.H.and WALTERS, M.C. (1988c). Aspects of the injuriousness of the maize stalkborer, *Busseola fusca* (Fuller) (Lepidoptera:Noctuidae). *Bulletin of entomological Research* **78** : 101 - 110.

VAN RENSBURG, J.B.J., WALTERS, M.C. & GILIOMEE, J.H. (1988d). The comparative abundance and in-season distribution of larval infestations of *Busseola fusca* (Fuller)(Lepidoptera:Noctuidae) and *Chilo partellus* (Swinhoe)(Lepidoptera:Pyralidae) on maize. *Journal of the entomological Society of South Africa* **51(1)** : 138 - 140.

VAN RENSBURG, J.B.J. (1988e). Efficacy of cloethocarb for control of *Busseola fusca, Cicadulina* spp. and nematodes in maize. *Applied Plant Science* **2(2)** : 63 - 67.

VAN RENSBURG, J.B.J., WALTERS, M.C. & GILIOMEE, J.H. (1988f). Plant population and cultivar effects on yield losses caused by the maize stalk borer, *Busseola fusca* (Lepidoptera : Noctuidae). *South African Journal of Plant and Soil* **5(4)** : 215 -218.

WAHL, R.O. (1930). The Maize Stalk Borer. *Farming in South Africa* **53(4)** : 205 - 206.

WALKER, P.T. (1960a). The relationship between infestation by the stalk borer, *Busseola fusca*, and yield of maize in East Africa. *The Annals of applied Biology* **48(4)** : 780 - 786.

WALKER, P.T. (1960b). Insecticide studies on the maize stalk borer, *Busseola fusca* (Fuller), in East Africa. *Bulletin of entomological Research* **51** : 321 - 351.

WALKER, P.T. (1965). The distribution of loss of yield in maize and of infestations of the maize stem borer, *Busseola fusca* (Noctuidae) in East Africa. *Rijksfakulteit der landbouwetenschappen te gent mededelingen* **30(3)** : 1577 - 1587.

WALKER, P.T. & HODSON, M.J. (1976). Developments in maize stem-borer control in East Africa, including the use of insecticide granules. *The Annals of applied Biology* **84** : 111 - 114.

WALKER, P.T. (1977). Crop losses : some relationships between yield and infection. *Mededelingen Faculiteit Landbouwwetenschappen, Rijksuniversiteit, Gent* **42** : 919 - 926.

WALKER, P.T. (1981). The relation between infestation by Lepidopterous stem borers and yield in maize: Methods and results. *EPPO Bulletin* **11(2)** : 101 - 106.

WALL, P.C. (1967). Some aspects of the life cycle and habits of the maize stalk borer *Busseola fusca* (Fuller) and the yield depression it caused in maize. *Special study presented towards the degree of B.Sc. Agriculture at the University College of Rhodesia and Nyasaland, University of London.*

WALTERS, M.C. (1974). Mechanisms of resistance to insects and application to maize stalk borer. *Proceedings of the 1st South African Maize Breeding Symposium.*

WALTERS, M.C. (1975). Evolution in tillage techniques and impact on entomological research, with special reference to the maize stalkborer, *Busseola fusca* (Fuller). *Proceedings of the 1st Congress of the entomological Society of South Africa* : 235 - 244.

WALTERS, M.C. & DRINKWATER, T.W. (1975). Preliminary studies on the application of systemic insecticides to the soil for the control of the maize stalk borer, *Busseola fusca* (Fuller) (Lep.: Noctuidae). *Phytophylactica* **7** : 121 - 124.

WEAVING, A.J.S. (1964). A preliminary assessment of the effectiveness of pyrethrum against the stalk borer *Busseola fusca* (Fuller) in the field in Kenya. *Bulletin of entomological Research* 55 : 565 - 572.

WIDSTROM, N.W. & HAMM, J.J. (1969). Combining abilities and relative dominance among maize inbreds for resistance to earworm injury. *Crop Science* 9 : 216 - 219.

WIDSTROM, N.W., WISEMAN, B.R. & McMILLIAN, W.W. (1972). Resistance among some maize inbreds and single crosses to Fall Armyworm injury. *Crop Science* 12 : 290 - 292.

WILLIAMS, D.P. & SANFORD, J.O. (1983). Corn and wheat in a double-cropping system. *Mississippi Agriculture and Forestry Experimental Station Research Report* 8 : 11.

WISEMAN, B.R., DAVIS, F.M. & CAMPBELL, J.E. (1980). Mechanical infestation device used in Fall Armyworm plant resistance programmes. *The Florida Entomologist* 63(4) : 425 - 432.

J. ent. Soc. sth. Afr. 1985
Vol. 48, No. 1, pp. 113–119

The effect of different maize genotypes on the maize stalk-borer, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae), feeding in whorl tissue.

by

M. R. BARROW

Pioneer Seed Company (Pty.) Ltd., P.O. Box 19, Greytown, 3500

Different amounts of leaf damage were caused to several maize genotypes by stalk-borer larvae feeding in whorl tissue. The extent of damage was correlated with the mean larval biomass/plant, which varied in the different maize genotypes. This variation is ascribed to two resistance factors: the first is thought to be a short-lived, but effective resistance factor in the whorl tissue which either kills or repels early instar larvae, resulting in fewer larvae feeding in those plants, while the second, operative for most of the larval feeding period in the whorl, may retard development and hence mass gain of larvae.

INTRODUCTION

Damage caused by first generation stalkborer larvae, *Busseola fusca* (Fuller), feeding in the whorl tissue of maize can be severe, necessitating the application of expensive control measures. If farmers had available maize hybrids that showed partial or complete resistance to stalk-borer, the financial savings would be of great benefit to them and the maize industry.

Previous attempts to breed hybrids resistant to maize stalk-borer in South Africa relied on natural pest infestations in the field. The first published attempt was by Du Plessis and Lea (1943), who stated that "significant differences occur in the degree of stalk-borer infestation of various varieties planted at the same time". However, they ascribed the differences to varietal rates of growth, and concluded that there was no resistance to stalk-borer in the varieties studied. Investigation of resistance to the maize stalk-borer resumed in 1953 but was discontinued 4 years later, as similar conclusions were reached, except that the differences in plant damage were ascribed to the use of natural pest infestations (Walters, 1974). Kühn (1978) investigated the amounts of damage caused to several homozygous maize cultivars (inbreds) by larvae resulting from natural oviposition in the field. Variable results were obtained due to moth preference, escapes, and differential numbers of eggs laid on the plants. Selection of resistant germ-plasm under such conditions has tended to be unreliable.

Elsewhere in Africa, no known resistance breeding has occurred, but in Nigeria, Usua (1968) investigated the yield loss due to stalk-borer, resulting from artificially infested maize. Using a small paintbrush, larvae were applied to the plant whorl.

Walker and Hodson (1976), working on yield losses in East Africa, also applied first instar larvae with a paintbrush to the whorl. Mihm *et al.* (1978), working in Mexico, developed a "Bazooka" which is a mechanical device for manually dispensing a mixture of maize meal and first instar lepidopterous larvae into the whorl. This method was used by the author of this paper, since it allows for controlled infestation of the maize plants with stalk-borer larvae.

Research carried out by the author during the past four seasons with artificially infested maize, using laboratory-reared larvae, provided the results reported here.

MATERIALS AND METHODS

The eleven maize inbreds chosen for the experiment had previously been screened with many others for resistance to first instar *B. fusca* larvae, and their selection was designed to give a wide range of leaf damage. The experiment was carried out in a commercial maize field. The seeds were planted on 4.11.82. This date was chosen so that the plants would not be at an attractive stage in November and December when the wild population of *B. fusca* moths was ovipositing, with the result that no natural oviposition was observed on any of the experimental plants.

The experimental plants were planted using a randomized complete block design, with split plots and 4 replications. Each inbred row contained 10 plants spaced 20 cm apart, giving a total of 80 plants for each inbred; each row had 20 kernels planted initially, and was then thinned to 10 plants/row when the plants were 10 cm tall. This gave a population of 25 000 plants/ha. All plants were infested thirty-one days post-emergence with approximately 21 larvae (mean of 20,8 larvae/plant over the whole trial) applied with a "bazooka" on the 15th December 1982.

Leaf damage was assessed after 21 days of larval feeding, by visually rating the damage on each plant on a 1 to 5 scale (1 = very little damage, 5 = severe damage). Of the 80 plants of each inbred line, 40 were removed from the field after 15 days feeding, and the remaining 40 plants removed after 25 days feeding. The whorl of each plant was unrolled and the larvae feeding therein were counted and weighed.

The data were analysed on a Univac computer of the University of Natal, with the Genstat system of Rothamsted Experimental Station, U.K., using the analysis of variance for a split plot experimental design for mean larval number and mass, and total biomass of larvae/plant.

RESULTS AND DISCUSSION

The reactions of maize plants exposed to stalk-borer attack can be measured in several ways. Kühn (1978) rated leaf damage, dead plants and ear damage, and various researchers in the U.S.A. have evaluated the insect damage on maize by rating leaf damage, stem tunnelling, number of holes per plant, stalks girdled, ear damage, yield loss and stunting (e.g. Starks *et al.* (1982) on the South Western Cornborer; Davis (1980) on the Fall Armyworm; Guthrie (1981) on the European Cornborer). Of all these methods, leaf damage rating is the quickest field method of damage assessment. The major objective of this experiment was to determine if differences in leaf damage caused to maize genotypes by stalk-borer larvae could be related to plant resistance to borer.

Leaf damage was rated in this experiment on a scale of 1 = very little damage to 5 = severe leaf shredding. There were significant differences between the leaf damage ratings of the different inbreds and the data is summarized in Table 1.

TABLE 1. Mean leaf damage ratings for each maize inbred, after 21 days feeding by maize stalk-borer larvae.

MAIZE INBRED										
Fo3	F23	D57	D50	Fo8	D55	M23	D53	D54	K11	S56
1,48	1,53	1,83	1,94	2,05	2,34	2,63	2,92	2,93	3,20	3,71
a*	ab	ab	ab	bc	bcd	de	ef	ef	f	g

*Means followed by the same letter are not significantly different (p = 0,05).
L.S.D. (5%) = 0,38 C.V. = 9,7%

No plants were rated 5, and no deaths occurred. This contrasts markedly with Usua's (1968) work on *B. fusca*, where he infested maize plants 45–60 cm tall with 1–5 larvae/plant, and recorded "dead heart" damage within "a few days" after infestation. Ingram's (1958) work on *B. fusca* reported that 5 larvae/plant did no appreciable damage. With the results from the present paper illustrating the various susceptibilities of different maize genotypes, it is evident that Usua must have been working with an extremely susceptible genotype or have infested the plants with mature larvae. The various conflicting reports of the amounts of damage caused by stalk-borer to maize were obviously due to uneven oviposition as mentioned by various researchers, and due to the different susceptibilities of the maize plants used in the experiments.

To determine the effects of the maize genotypes on stalk-borer larvae, larvae were removed from the plants at two feeding intervals, after fifteen and twenty-five days and were counted and weighed.

Table 2 indicates that there are significant differences between the numbers of larvae recovered from each inbred and also in the numbers of larvae recovered after the two sampling periods. The interaction between the inbreds and sampling period was also highly significant ($F = 3,8$, $p < 0,01$).

TABLE 2. An interaction table showing the mean number of larvae/plant removed from 11 maize inbreds, after 15 and 25 days feeding.

FEEDING PERIOD	MAIZE INBREDS										
	Fo8	D57	D55	D50	F23	K11	Fo3	D53	S56	M23	D54
15 Days	1,08	2,30	2,05	3,35	3,30	3,52	3,37	4,07	3,87	4,57	3,47
25 Days	2,62	2,25	3,17	2,37	3,60	3,90	4,12	3,95	4,67	3,97	5,95
MEAN	2,23	2,27	2,61	2,86	3,45	3,71	3,75	4,01	4,27	4,27	4,71
	a*	a	a	ab	bc	cd	cd	cd	de	de	e

L.S.D. (5%)	Main effect	Interaction
Feeding period	0,34	0,96
Inbreds	0,66	0,95

*Means followed by the same letter are not significantly different (p = 0,05)

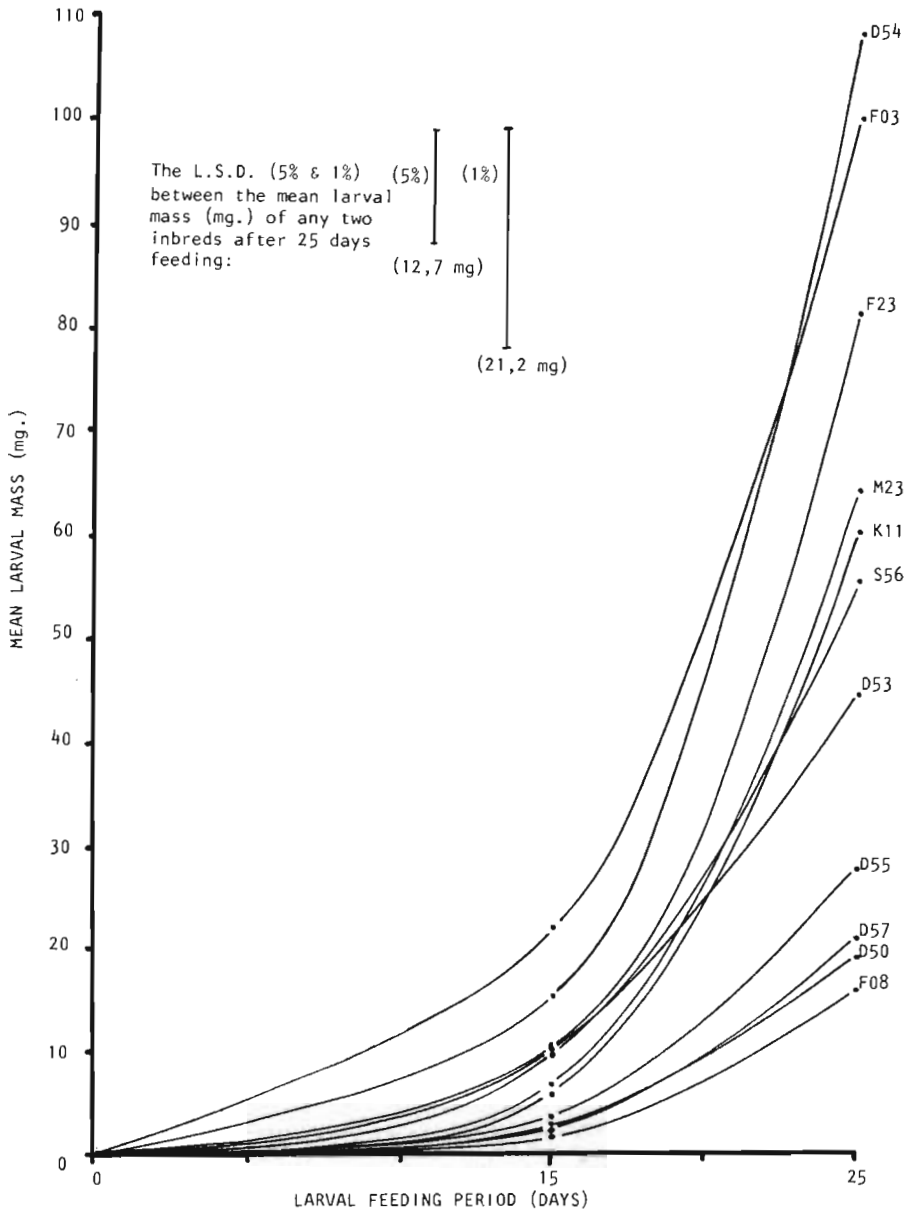


Fig. 1. Mean mass (mg) of *B. fusca* larvae feeding on 11 maize inbreds, removed after 15 and 25 days of feeding.

It appears that there is a resistance factor present in some inbreds which operates to reduce larval numbers within the first fifteen days of feeding. From an initial infestation of a mean of 20,8 larvae per plant, larval numbers decreased to a mean of 1,08 larvae/plant for Fo8, increasing gradually to the highest number of 4,57 larvae/plant for M23. After 25 days feeding there was no further reduction in larval numbers, indicating that the resistance factor affecting larval numbers operates only within the first fifteen days of larval feeding. Data from unpublished research indicates that this factor operates within the first 4 days of feeding.

In addition to counting the numbers of larvae/plant, the larvae were also weighed. There were significant differences between the mean larval masses recovered from each inbred and also in the mean mass of larvae recovered after the two sampling periods ($F = 414{,}6$, $p < 0{,}01$). The interaction between the inbreds and sampling periods was also highly significant ($F = 12{,}6$, $p < 0{,}01$).

Fig. 1 indicates that there was a rapid gain in larval mass on certain of the maize inbreds (F23, Fo3 and D54) during the 10 day period after the fifteen day sampling; in others (Fo8, D50, D57 and D55) there appears to be a resistance factor retarding larval development. As larvae feed for about thirty days, the resistance is long lasting.

It appears from the data presented that two resistant factors are active in some maize genotypes against leaf-feeding stalk-borer larvae – one factor limits the number of larvae surviving in plants, and the other retards their mass gain. There were highly significant differences between inbreds with regard to the mean larval biomass/plant and also between the mean larval biomass/plant recovered after the 2 sampling periods ($F = 362{,}6$, $p < 0{,}01$). The interaction between the inbreds and sampling periods was also highly significant ($F = 22{,}5$, $p < 0{,}01$) (Fig. 2). It is clear from Fig. 2 that the inbreds Fo8, D57, D50 and D55 supported a very low larval biomass after 25 days feeding (44,6 to 91,5 mg/plant); there was an intermediate group of D53, K11, M23, S56 and F23 (177,6 to 295,4 mg/plant), and two, Fo3 and D54, had the highest larval biomass/plant of 420,4 mg and 648,2 mg/plant respectively.

All inbreds except Fo8, D50 and D57 showed highly significant gains in larval biomass during the 10 day period between 15 and 25 days. D57 showed a significant gain in larval biomass, and Fo8 and D50 showed no significant gain in larval biomass. These data confirm that these three inbreds evidently have a long lasting resistance affecting larval mass gain.

Two anomalies appear in Figure 2, the inbreds Fo3 and F23 show a surprisingly high larval biomass, but the lowest leaf damage (Table 1). It is surmised that the nutritional status of these two inbreds is very high, allowing larvae to consume the same amount of whorl tissue as larvae feeding in other resistant inbreds (D57, D50 and Fo8 for example), yet to gain mass far quicker. If these two inbreds are excluded from a correlation analysis between mean larval biomass/plant after fifteen days feeding and leaf damage ratings after 21 days feeding, a highly significant correlation occurs ($r = 0{,}65$, $p < 0{,}01$) between these data.

Obviously plants having a low nutritional status can be considered as having resistance to stalk-borer larvae. Once nutrition becomes a limiting factor, it can be equated with resistance, but this resistance may not necessarily be due to the occurrence of an antibiotic chemical, as is the case with the European Cornborer, where resistance is due to the chemical 2,4-dihydroxy-methoxy-1,4-benzoxazin-one which affects larval development (Robinson *et al.*, 1982).

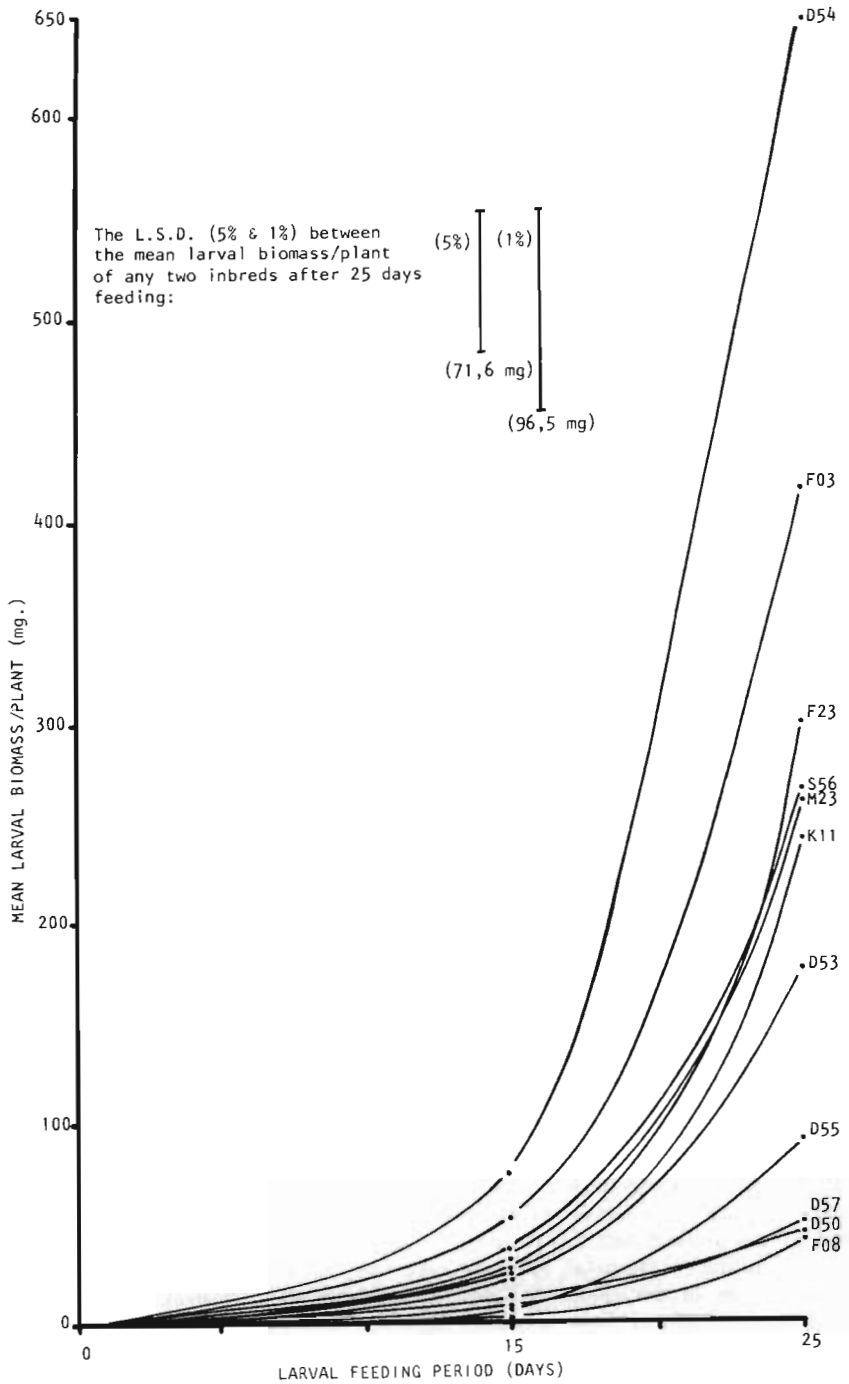


Fig. 2. Mean larval biomass/plant (mg) of *B. fusca* larvae removed from 11 maize inbreds, after 15 and 25 days of feeding.

From my results it appears that the extent of leaf damage by *B. fusca* larvae, their survival and development are affected by the maize inbred on which they are placed. This may be due to the presence in the maize of either one or two resistance factors: a short-lived, but effective, factor reduces larval numbers, and a second longer-lasting factor retards larval development. The resulting reduction in numbers of larvae present causes less damage than that of larvae feeding in maize genotypes deficient in these factors. However, as *B. fusca* is such a destructive pest of maize, even when present in low numbers, the resistance reported here is not of a strong enough nature, nor of a common enough occurrence, to incorporate immediately into South African maize hybrids (which usually have four inbred parents). A recurrent selection program (which recombines the more resistant selections in a breeding program over several years) is possibly the only way of increasing the resistance to a sufficiently high level to be of use in the production of stalk-borer resistant maize hybrids.

REFERENCES

DAVIS, F. M. 1980. Fall armyworm plant resistance programs. *Florida Entomologist* **63(4):** 420–424.

DU PLESSIS, C. & LEA H. A. P. 1943. The maize stalk-borer, *Calimistis fusca* (Hmps). *Department of Agriculture of Southern Africa Bulletin* **238,** 51 pp.

GUTHRIE, W. D. 1981. Maize Whorl stage resistance to the first four instars of European Cornborer larvae (Lepidoptera: Pyralidae). *Journal of the Kansas Entomological Society* **54(4):** 737–740.

INGRAM, W. R. 1958. The Lepidopterous stalk-borers associated with Gramineae in Uganda. *Bulletin of Entomological Research* **48:** 367–383.

KÜHN, H. C. 1978. Selection for resistance against the maize stalk-borer *(Busseola fusca). Proceedings of the 3rd South African Maize Breeding Symposium:* 84–86.

MIHM, J. A. PEAIRS F. B., & ORTEGA, A. 1978. New procedures for efficient mass production and artificial infestation with Lepidopterous pests of maize. *CIMMYT Review.* 138 pp.

ROBINSON, J. F., KLUN J. A., GUTHRIE, W. D., AND BRINDLEY, T. A. 1982. European Cornborer (Lepidoptera: Pyralidae) leaf feeding resistance: DIMBOA bioassays. *Journal of the Kansas Entomological Society* **55(2):** 357–364.

STARKS, K. J., BURTON, R. L., WILSON, R. L., & DAVIS, F. M. 1982. Southwestern cornborer: Influence of planting dates and times of infestation on damage to corn, pearl millet and sorghum. *Journal of Economic Entomology* **75:** 57–60.

USUA, E. J. 1968. Effect of varying populations of *Busseola fusca* larvae on growth and yield of maize. *Journal of Economic Entomology* **61:** 375–376.

WALKER, P. T. & HODSON, M. J. 1976. Developments in maize borer control in East Africa, including the use of insecticide granules. *Annals of Applied Biology* **84:** 111–114.

WALTERS, M. C. 1974. Mechanisms of resistance to insects and application to maize stalk-borer. *Proceedings of the First South African Maize Breeding Symposium:* 83–87.

Accepted 8 October 1984.

J. ent. Soc. sth. Afr. 1987
Vol. 50, No. 2, pp. 291–298

The effect of first generation maize stalkborer, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae), on yield of different maize genotypes

by

M. R. BARROW

Pioneer Seed Company (Pty.) Ltd.; P.O. Box 19, Greytown, 3500

Differences in resistance to the larvae of *B. fusca* feeding in whorl tissue have been identified in certain maize genotypes, but there are no data on the effectiveness of this resistance in reducing yield loss. To determine whether first generation stalkborer larvae feeding in the whorl of different maize genotypes resulted in different levels of yield loss, 11 homozygous maize cultivars (inbred lines) were each artificially infested with approximately 20 first instar larvae. Highly significant differences in leaf damage, stem damage and stunting were observed among the maize genotypes. Yield potentials of the genotypes also varied significantly under stalkborer attack, with reductions in yield ranging from 38% to 100%. This yield loss was significantly correlated with leaf damage ($r = +0{,}39$, $p < 0{,}01$), but showed significantly higher correlation with the amount of stem boring damage ($r = +0{,}56$, $p < 0{,}01$). Plants that showed severe stem boring also showed significant reductions in plant height ($r = +0{,}73$, $p < 0{,}01$). It is concluded that field selection for resistance to *B. fusca* should rely on leaf damage recorded after 24 days feeding and visual assessment at harvest of plant height reduction and yield.

INTRODUCTION

The maize stalkborer is generally considered the most widespread and most destructive of all insects attacking maize in Africa (Walters *et al.* 1980; Rose 1962; Smithers 1960; Anderson and Wessels 1959). For decades research has centred around chemical control of the borer (Walker and Hodson 1976; Walker 1961, 1972; Du Plessis and Lea 1943; Malley 1920; Jack 1917), and attempts have been made to calculate the extent of damage due to the uncontrolled feeding of the larvae in maize plants. These experiments have centred around natural infestations which varied considerably, as did the ages of the various crops at the time of their infestation. Stalkborer infestations ranged from 14% with a yield loss of only 9,8% (Anon 1975), to 49% stalkborer infestation, with a yield loss of 37% in untreated plots (Walker 1960). Swaine (1957) recorded 22% damaged plants in untreated lands, and harvested 83% more grain from uninfested plants than from infested plants. As will be discussed in this paper, the amount of time spent by the stalkborer feeding in the stem has a direct bearing on yield loss.

In addition to the abovementioned, attempts have been made over the years

to breed for resistance against the borer (Kühn 1978; Walters 1974; Du Plessis and Lea 1943).

In a recently published paper, Barrow (1985) determined that different amounts of leaf damage caused to several maize genotypes by artificially-applied first generation stalkborer larvae feeding in whorl tissue, were correlated with the mean larval biomass/plant, which varied in the different maize genotypes. This variation in larval biomass was ascribed to two resistance factors: the first is a short-lived, but effective, resistance factor in the whorl tissue which either kills or repels early instar larvae, resulting in fewer larvae feeding in those plants possessing this resistance; the second, which is operative for most of the larval feeding period in the whorl tissue, retards development, and hence mass gain, of larvae.

However, the infestation does not terminate in the whorl tissue; after a feeding period of between 21–35 days, larvae leave the whorl tissue (either when the tassel emerges and forces the larvae out of the whorl, or when larvae reach the pre-pupal stage) and bore into the stems of maize plants, causing considerable damage to stem tissue until pupation occurs. As stem boring restricts the flow of nutrients and water to the ear of the plant, serious yield losses can occur. As the larvae change from feeding on leaf tissue to stem tissue, it is probable that they encounter a totally new set of plant substances, with resistance factors different to those of the leaf tissue.

The question then arises: If certain maize genotypes show a resistance reaction to stalkborer feeding in the whorl, is that leaf resistance effective throughout the larval feeding period in both leaf and stem tissue, and are the yield of these maize genotypes affected differently? This paper attempts to answer that question.

MATERIAL and METHODS

The major objective of this experiment was to determine whether the effect on larvae of resistance factors present in the leaf tissue is sufficient to reduce yield losses. The same eleven maize inbred lines used previously by the author (Barrow 1985) were planted on 8/11/83 in a commercial maize field receiving the same fertilizer, irrigation and cultural practices as the commercial crop. No natural oviposition by wild female moths of *B. fusca* was observed, as the first generation moth flight had terminated by the time the plants were at an attractive stage, and the second generation flight had not yet commenced.

The experimental plants were planted in a completely randomized block design with two treatments which were split and four replications. The whole plot treatments were the infestation levels (0 and 20 larvae/plant), and the subplot treatments were the 11 inbred lines. Each replicate of an inbred line consisted of two rows, with each row containing 10 plants spaced 20 cm apart, giving a total of 80 plants for each inbred line; each row had 20 kernels planted initially and was then thinned to 10 plants/row when the plants were 10 cm high. This gave a final population equivalent to 25 000 plants/ha. As larvae migrate out of the whorl just prior to pupation, and may enter the stems of nearby plants to pupate, barrier rows were densely planted between treatment rows, at 10 cm spacing, in order to prevent larvae moving into other treatments and confusing the stem damage assessment. All plants were infested 29 days post emergence with approximately 20 first instar larvae (mean of 19,7 larvae/plant over the whole trial) applied down the funnel by means of a mechanical dispenser called the 'Bazooka' (Mihm *et al* 1978).

Leaf damage was assessed by visually rating the extent of leaf damage on

each plant on a scale of 1 to 5 (1 = very little damage, 5 = severe damage) after 24 days of larval feeding. The ears were harvested and shelled on a plot basis (20 plants), the grain weighed and the moisture content determined. The final yields are expressed as mean yield in g per plant, adjusted to 12,5% moisture mass. The heights of infested plants were also compared with the heights of the uninfested plants by visually rating the stunting of the infested plants on a scale of 1 to 5 (1 = very little stunting, 5 = severe stunting). The stems of all the plants split immediately after harvest, and internal damage to each plant was rated on a scale of 0 to 9 (0 = nil damage, 9 = severe damage).

The date were analysed on a Univac computer of the University of Natal, with the Genstat system of Rothamsted Experimental Station, U.K., using the analysis of variance for a split plot experimental design for leaf damage, stem damage, percentage height loss and percentage yield loss between the infested and uninfested rows of each inbred line.

RESULTS AND DISCUSSION

It was shown by Barrow (1985) that the extent of leaf damage caused by *B. fusca* larvae was strongly correlated to the mean larval biomass per plant after 15 days feeding, and that recorded differences in larval biomass were due to the presence in the maize of either one or two resistance factors that affected larval numbers and larval development.

Significant differences ($F = 25{,}31$ $p < 0{,}01$) were observed between the leaf damage ratings of the different inbred lines (Table 1).

Data recorded in 1982 are included for comparison and although the damage was slightly more severe in the 1983 infestation, there is acceptable correlation between the season's ratings, and a useful range of damage ratings occurred.

TABLE 1: Mean leaf damage ratings for 11 maize inbreds, after 24 days feeding by maize stalkborer larvae (Two seasons' data given; 1982 data from Barrow 1985).

	MAIZE INBRED										
	F23	Fo3	D57	D50	D55	Fo8	D54	M23	D53	K11	56
1983	1,84	1,99	2,01	2,38	2,59	2,71	3,26	3,60	3,67	3,69	4,00
	a*	ab	ab	bc	c	c	d	d	d	d	e
1982	1,53	1,48	1,83	1,94	2,34	2,05	2,93	2,63	2,92	3,20	3,71
	pq**	p	pq	pq	qrs	qr	tu	st	tu	u	v
Mean:	1,68	1,73	1,92	2,16	2,47	2,38	3,09	3,11	3,29	3,45	3,85

Means in each row followed by the same letter are not significantly different (P = 0,05).
** 1982 L.S.D. 5% = 0,38 C.V. = 9,7%
* 1983 L.S.D. (5%) = 0,45 C.V. = 10,8%

TABLE 2: Mean height reduction ratings of 11 inbreds recorded at tasseling, caused by stalkborer larvae boring into stem tissue.

MAIZE INBRED										
Fo8	Fo3	D57	D55	F23	D54	D50	M23	D53	K11	56
1,25	2,25	2,25	2,50	3,00	3,00	3,25	3,50	4,75	5,00	5,00
a	b	b	bc	bc	bc	bcd	cd	e	e	e

Means followed by the same letter are not significantly different (p = 0,05).
L.S.D. (5%) = 1,00 C.V. = 24,3%

The effect of stalkborer on plant growth was assessed by rating the difference in height between the infested plants and uninfested plants of the same inbred line (Table 2).

Significant differences were apparent between the inbred lines with regard to stunting, with lines Fo8, Fo3 and D57 showing very little stunting due to stalkborer damage, and D53, K11 and 56 showing severe stunting. With the exception of Fo8 (which had intermediate leaf damage but very little stunting) there was good correlation between leaf damage ratings and stunting ($r = + 0,53$, $p < 0,01$ – if the value for Fo8 is deleted, $r = + 0,74$ ($p < 0,01$). During the leaf feeding period (21–35 days) no measurements of height reduction were recorded, but it was evident that little, if any stunting was occuring. Stunting was therefore not causally related to leaf feeding, but was due to stem boring activity which occurred as early as 21 days post infestation in some inbreds, and as late as 35 days in others. Stem damage ratings were taken per plant at harvest, and are presented in Table 3.

There were significant differences between the stem damage ratings of the inbred lines ($F = 4,79$, $p < 0,01$), and the correlation coefficient between stem and leaf damage was significant in all inbreds ($r = + 0,54$, $p < 0,01$). An exception was the inbred line F23, which showed very little leaf damage (1,84 rating), yet showed substantial stem boring (6,45 rating). This phenomenon is possibly due to the stem tissue of F23 having a low nutritional value, resulting in larvae having to consume a large amount of tissue in order to reach the pre-pupal stage.

TABLE 3: Mean stem damage ratings at harvest of 11 inbreds infested with stalkborer.

MAIZE INBRED										
Fo3	D57	Fo8	D55	D50	M23	D54	F23	56	D53	K11
3,54	3,73	4,49	4,63	5,33	5,88	6,00	6,45	6,74	6,82	8,30
a	ab	abc	abcd	bcde	cbc	cdc	dc	e	e	f

Means followed by the same letter are not significantly different (p = 0,05).
L.S.D. (5%) = 1,90 C.V. = 23,5%

Further research into stem resistance/susceptibility will shed light on the interpretation of differences in stem damage observed in different maize genotypes.

Some inbred lines were severely stunted (56, D53 and K11), and these also suffered severe stem damage. The least stunted inbred lines (F08, F03, D57 and D55) also showed the least stem damage. The correlation coefficient between stunting and stem damage was highly significant ($r = + 0{,}73$, $p < 0{,}01$), and there is a highly significant causal relationship between stem boring damage and height reduction, and as will be described later, the yields of the worst stem damaged inbred lines were also significantly the most depressed. Larvae feed in whorl tissue until either the emergence of the tassel forces them to migrate out of the funnel and into the stem to continue feeding, or until they have reached the pre-pupal stage, when they move out of the whorl into the stem to pupate, irrespsective of whether the tassel has emerged. In case larval movement out of the funnels was due to early tassel emergence, the times of tassel emergence were noted on all inbred lines and are shown in Table 4, along with the stem damage ratings.

TABLE 4: Age of 11 inbreds at tassel emergence and extent of stem damage caused by stalkborer larvae.

Inbred[1]	Plant age[2] to tasseling	Stem damage ratings[3]	Days from infestation to 50% tasseling
F03	49	3,54 a	21
D57	68	3,73 ab	37
F08	71	4,49 abc	44
D55	63	4,63 abcd	35
D50	70	5,33 bcde	42
M23	63	5,88 cde	35
D54	49	6,00 cde	21
F23	49	6,45 de	21
56	70	6,74 e	42
D53	70	6,82 e	42
K11	73	8,30 f	45

1. Inbreds arranged in order of increasing stem damage.
2. Days from plant emergence to 50% tassel emergence.
3. Mean ratings followed by the same letter are not significantly different ($p = 0{,}05$).

It is evident that early tassel emergence, which forces larvae out of the whorl, is not causally related to severe stem damage. The inbred lines F03 and F23 both had 50% tassel emergence 21 days after infestation, yet had significantly different stem damage ratings of 3,54 and 6,45, and as both these inbred lines showed similar leaf damage ratings, the higher stem damage rating of F23 was not due to larger larvae moving out of the whorl of F23. Two other inbreds, F08 and K11, had tassels emerge 44 and 45 days respectively after infestation, yet had significantly different stem damage ratings of 4,49 and 8,30. It is probable that differences in susceptibility or resistance of the stem tissue were responsible for the widely differing stem damage ratings in those inbred lines which had similar leaf damage ratings, and had stem boring commencing at the same time.

TABLE 5: Mean grain yields (g/plant) of 11 inbreds, taken from infested and uninfested plots.

Inbred[1]	Mean yield/plant (g) Uninfested	Infested	% Yield[2] Reduction
D54	46,69	28,68	38,57 a
D55	38,26	21,50	43,80 ab
Fo8	52,76	24,84	52,92 ab-c
D57	66,55	31,04	53,36 ab-c
Fo3	34,08	13,51	60,36 bc
D50	54,75	17,91	67,29 cd
M23	60,62	9,44	84,43 de
F23	47,46	6,92	85,42 de
D53	40,77	3,14	92,99 e
K11	40,46	2,92	92,78 e
56	39,91	0,00	100,00 e

1. Arranged in order of increasing yield loss.
2. Means followed by the same letter are not significantly different (p = 0,05).
L.S.D. (5%) = 19,70 C.V. = 19,6%

The effect on yield, of larvae feeding in leaf and stem tissue of the inbred lines, was investigated by comparing the mean yields of infested and uninfested plants (Table 5).

There were significant differences between the yields of infested and uninfested plants of all inbred lines and significantly different amounts of yield loss between inbred lines. The lowest yield loss occurred in D54 (38,57% loss) and the highest yield loss occurred in 56 (100,00%), an inbred line whose plants were severely stressed and stunted and which produced very few ears, none of which bore any grain. The correlation coefficient for yield and leaf damage was significant ($r = + 0,39$, $p < 0,01$) but low, as expected, as very little leaf area is lost through larval feeding which therefore probably has very little effect on yield. However, once larvae move into the stem tissue, severe damage can result in loss of water and nutrient flow, hence the correlation between yield and stem damage ($r = + 0,56$, $p < 0,01$).

From these results it is evident that although previous research has shown leaf damage ratings to be a quick and efficient field method of selection for resistance to first generation stalkborer, it reflects only the resistance factors present in the leaf whorl. Apart from feeding on leaf tissue, *B. fusca* larvae may also feed for a short time (5–10 days) on the enclosed tassel, and also for varying periods in stem tissue. Both sites probably have varying degrees of susceptibility or resistance to the larvae and affect larval development and hence damage resulting in yield loss. In answer to the question as to the efficacy of leaf resistance providing protection against yield loss, it is evident that resistance factors present in the stem tissue and duration of the larval feeding period in stem tissue are additional critical components in a complex interaction of several factors affecting yield.

The splitting of stems is a time-consuming task and impractical under field conditions where thousands of plants have to be evaluated each season in a breeding

programme for resistance to maize stalkborer. Yield is an easy component to assess at harvest, and so is stunting of plants if uninfested control plants are available. Because of the good correlation between leaf and stem damage, and between stem damage, stunting and yield, a quick and effective field method of selection for resistance to whorl-feeding *B. fusca* larvae should include a recorded rating of leaf damage after 24 days feeding in the whorl, a comparison of stunting between infested and uninfested plants of the same genotype, and an assessment of *per se* yield. Plants with low leaf damage, little stunting, and good yield are the obvious choices for selection. It is probable that certain genotypes would show high leaf damage coupled with low stem damage and good yield; however, for the presence of resistance to *B. fusca* in maize hybrids to be appreciated by the farmer growing such a hybrid, it would be of paramount importance for the farmer to see reduced leaf damage, for this leaf damage is often the only criterion a farmer will consider as to the effectiveness of resistance to first generation stalkborer feeding in the whorl.

REFERENCES

ANDERSON, V. S. & P. P. M. WESSELS. 1959 The maize stem borer and its control in South Africa. *Höfchen-Briefe* **12:** 276–285.

ANON. 1975. Maize Stalkborer Research. *Plant Protection Research Institute Annual Report 74/75. Salisbury Rhodesia*, P37.

BARROW, M. R. 1985. The effect of different maize genotypes on the maize stalkborer, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae), feeding in whorl tissue. *Journal of the Entomological Society of Southern Africa* **48**(1): 113–119.

Du PLESSIS, C. & H. A. P. LEA 1943. The maize stalkborer, *Calamistis fusca* (Hmps). *Bulletin of the Department of Agriculture of South Africa* **238,** 51 pp.

JACK, R. W. 1917. The maize stalkborer *Calamistis fusca* (Hmpson). *The Bulletin of the Department of Agriculture of Southern Rhodesia* **276,** 12 pp.

KÜHN, H. C. 1978. Selection for resistance against the maize stalkborer *(Busseola fusca). Proceedings of the 3rd South African Maize Breeding Symposium;* 84–86.

MIHM, J. A., F. B. PEAIRS, & A. ORTEGA. 1978. New procedures for efficient mass production and artificial infestation with Lepidopterous pests of maize. *CIMMYT review:* 138 pp.

MALLEY, C. W. 1920. The maize stalkborer *Busseola fusca* (Fuller). *The Department of Agriculture of South Africa Bulletin* **3:** 111 pp.

ROSE, D. J. W. 1962. Pests of maize and other cereal crops in the Rhodesias. *The Rhodesian Agricultural Journal Bulletin.* 2163.

SMITHERS, C. N. 1960. Some recent observations on *Busseola fusca* (Fuller), (Lepidoptera: Noctuidae) in Southern Rhodesia. *The Bulletin of Entomological Research* **50:** 809–819.

SWAINE, G. 1957. The maize and sorghum stalkborer, *Busseola fusca* (Fuller), in peasant agriculture in Tanganiyika territory. *The Bulletin of Entomological Research* **48:** 711–722.

WALKER, P. T. 1960. Insecticide studies on the maize stalkborer, *Busseola fusca* in East Africa. *The Bulletin of Entomological Research* **51:** 321–351.

———— 1961. The distribution of granules by a commercial hand operated distributor and their retention by maize plants. *The Journal of Agricultural Engineering Research* **6:** 112–118.

———— 1972. The effect of the physical properties of granules on their insecticidal performance. *Pesticide science* **3:** 805–809.

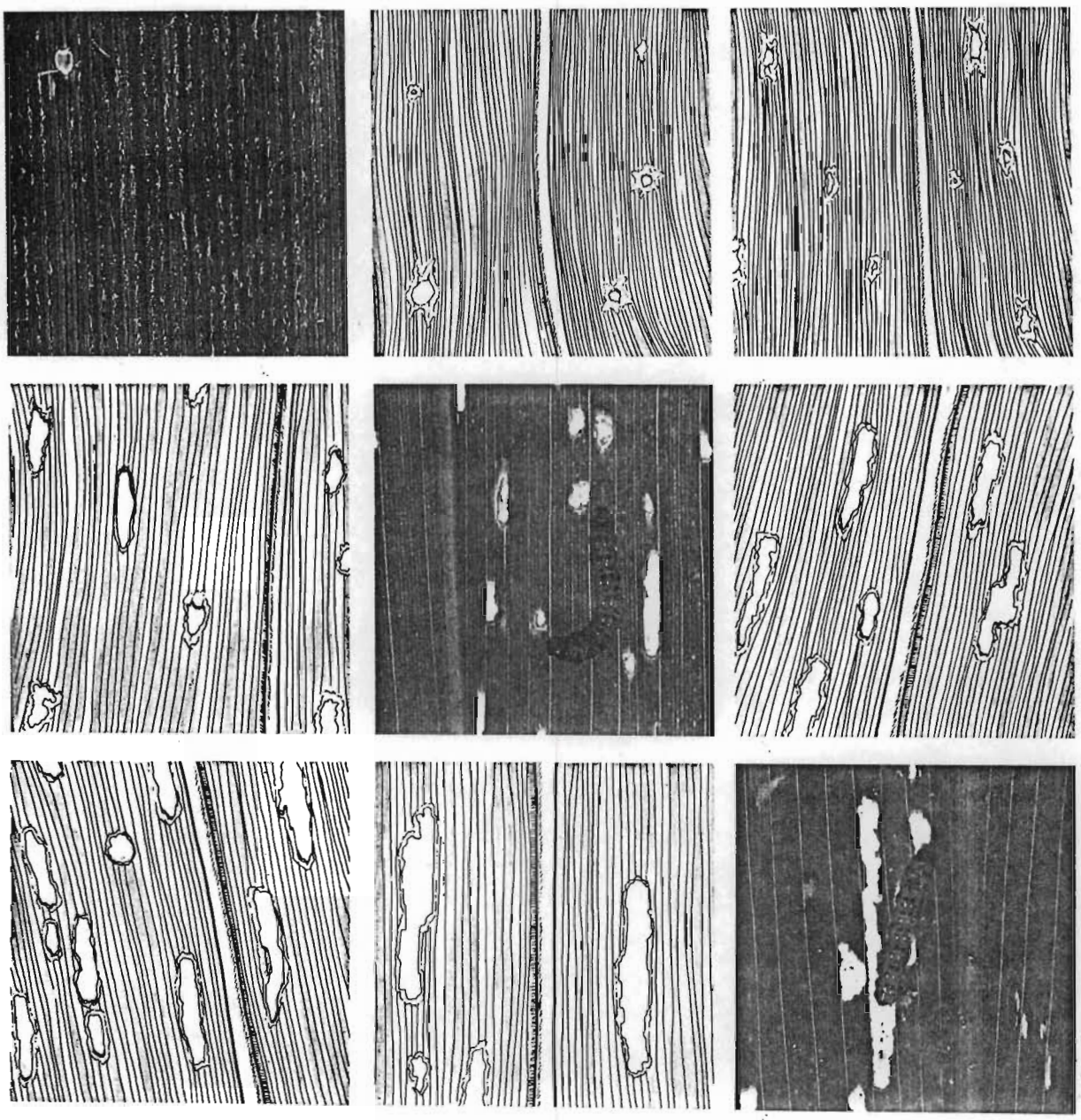
WALKER, P. T. & M. J. HODSON. 1976. Developments in maize stemborer control in East Africa, including the use of insecticide granules. *The Annuals of Applied Biology* **84:** 111–114.

WALTERS, M. C., T. W. DRINKWATER, J. B. J. VAN RENSBURG, & L. BOSHOFF. 1980. The maize stalkborer. *The Division of Agricultural Information, Pretoria, South Africa. "Maize D3/1980"*

WALTERS, M. C. 1974. Mechanisms of resistance to insects and application to maize stalkborer. *Proceedings of the First South African Maize Breeding Symposium:* 83–87.

Accepted 24 September 1986

Toward Insect Resistant Maize for the Third World



Proceedings of the International Symposium on Methodologies for Developing Host Plant Resistance to Maize Insects

CIMMYT, UNDP, GTZ, and USAID

The International Maize and Wheat Improvement Center (CIMMYT) is an internationally funded, nonprofit scientific research and training organization. Headquartered in Mexico, the Center is engaged in a worldwide research program for maize, wheat, and triticale, with emphasis on food production in developing countries. It is one of 13 nonprofit international agricultural research and training centers supported by the Consultative Group on International Agricultural Research (CGIAR), which is sponsored by the Food and Agriculture Organization (FAO) of the United Nations, the International Bank for Reconstruction and Development (World Bank), and the United Nations Development Programme (UNDP). Donors to the CGIAR system are a combined group of 40 donor countries, international and regional organizations, and private foundations.

CIMMYT receives core support through the CGIAR from a number of sources, including the international aid agencies of Australia, Austria, Brazil, Canada, China, Denmark, Finland, France, West Germany, India, Ireland, Italy, Japan, Mexico, the Netherlands, Norway, the Philippines, Spain, Switzerland, the UK, and USA, and from the European Economic Commission, Ford Foundation, Inter-American Development Bank, OPEC Fund for International Development, UNDP, and World Bank. CIMMYT also receives non-CGIAR extra-core support from Belgium, the International Development Research Centre, the Rockefeller Foundation, and many of the core donors listed above.

Responsibility for this publication rests solely with CIMMYT.

Correct Citation: CIMMYT. 1989. *Toward Insect Resistant Maize for the Third World: Proceedings of the International Symposium on Methodologies for Developing Host Plant Resistance to Maize Insects.* Mexico, D.F.: CIMMYT.

ISBN 968-6127-35-6

Screening and Breeding for Resistance to *Busseola fusca*

Mike Barrow, Pioneer Seed Company, Greytown, Republic of South Africa

Abstract

The general biology of Busseola fusca *and various methodologies necessary for a program of breeding for resistance to* B. fusca *are described. The winter collection and cold room storage of diapausing larvae, and procedures for handling pupae, moths, eggs, and larvae in the laboratory are described. Field infestation using a mixture of maize meal and first-instar larvae dispensed by a mechanical applicator is discussed. Plants are infested once at a height of about 35 cm with 16 to 22 larvae/plant. Leaf damage is rated after 21 to 25 days feeding on a scale of 1 = very little damage to 5 = severe leaf shredding. Note is also taken at harvest of stunting and grain yield. In the wide range of germplasm that has shown varying degrees of resistance, the expression of the resistance has been found to affect larval numbers (repellency and antibiosis) and growth rate (antibiosis) resulting in a lower larval biomass/plant with a concommitant reduction in yield loss. To be of commercial use, resistant inbreds must also be agronomically superior and must combine well with susceptible elite inbreds in order to produce competitive hybrids.*

Throughout the maize and sorghum growing areas of Africa, the maize stalk borer *Busseola fusca* (Fuller) is a major pest requiring the application of expensive chemical control measures in order to avoid severe crop losses (Seshu Reddy 1985; Revington et al. 1984; Kaufmann 1983a; Mlambo 1983; Egwuatu and Ita 1982; Walker 1981; Ogunwolu et al. 1981). Various attempts have been made to develop resistant maize cultivars as an alternative or addition to chemical applications, but with little success (Kuhn 1978; Walters 1974; du Plessis and Lea 1943).

Recently Barrow (1985) reported that different amounts of leaf damage were caused to several maize genotypes by *B. fusca* larvae feeding in whorl tissue, and that the extent of damage was correlated with the mean larval biomass/plant. This variation in mean larval biomass present in the different maize cultivars was ascribed to two resistance factors present in the leaves: the first is a short-lived, but effective resistance factor which either kills or repels early instar larvae, resulting in fewer larvae surviving, while the second, operative for most of the larval feeding period in the whorl, retards larval development and growth.

Investigation of resistance in maize to *B. fusca* was initiated at Pioneer's research station in Greytown Natal, South Africa, in 1977. The goal of incorporating resistance into commercial hybrids has necessitated the development and refinement of several key procedures, each of which is described below.

Basic Behavior and Biology

The interaction between *B. fusca* and the maize crop is basically the same as recorded for other Lepidopterous borers. The first-generation infestation develops from moths emerging in spring (October) from diapausing larvae overwintering in maize stalks. The moths are attracted over great distances to young maize plantings, where they oviposit within the leaf sheaths. Data recorded in Natal show that eggs are laid within the sheath of any one of the 5th to the 10th leaves on plants ranging in height from 26 to 75 cm, with a distinct preference by moths for plants between 26 and 45 cm tall (Table 1).

The number of eggs per batch ranged from 5 to 37, with the majority of egg batches (79%) containing 11 to 25 eggs (Table 2), considerably fewer than the maximum number of 300 (avg. 92) recorded by Kaufmann (1983b) in Nigeria, but comparable to the average of 22.1 eggs/batch found by van Rensburg (1981) in South Africa and 25.2 eggs/batch recorded by Usua (1968) in Nigeria.

As maize is planted extensively by the commencement of the mothflight, oviposition is widespread, not normally recorded as being more than 10% in any one field. However, first-instar larval dispersal can increase the percentage of plants infested to anywhere between 10 and 100%. The eggs hatch in 7 to 12 days depending on ambient temperature. The larvae remain at the oviposition site for a day or two, during which time they consume the eggshells, and then migrate up the stem and down into the funnel or disperse to adjacent plants. They feed in the funnel for periods of 25 to 35 days, depending primarily on ambient temperature, the age of the plant at infestation, and the time of tassel emergence.

If plants are young at infestation, larvae complete their development in the whorl, and then migrate out of the whorl and bore into the stem, where pupation occurs. Irrespective of how short a period the larvae have been feeding in the whorl, the emergence of the tassel forces them out of the whorl and into the still enclosed developing tassel, where they feed for a short while (approximately 10 days). As soon as the tassel emerges, the larvae bore into the stem, where they pupate after varying periods of stem feeding. Larvae feed successively on developing leaf tissue, tassel glumes, stalk, and finally stem tissue. The larvae are therefore exposed to a variety of food sources, each of which probably has a different nutritional status, and therefore a different effect on larval development. The larvae pupate in the stem after chewing a small perforated "window" in the outer stem tissue, which is pushed out later by the emerging moth.

The second-generation infestation takes place during the period from late January to early March, when late planted maize is generally at the tasseling stage. Moths are attracted from great distances to the youngest maize in the area, and the percentage of plants that have eggs laid on them can increase to 90%. Eggs are generally laid under leaf sheaths of the lower leaves, but will also be laid under the ear husk leaves. Larvae bore into the ear and stem, and commence feeding in these sites. In warmer areas, *B. fusca* may have a partial third generation, but the majority of larvae enter diapause in the autumn, spending the cold winter months in the plant stems, generally in the part just below ground level.

Source of Insects

In order to sustain a host plant resistance program involving mass infestation and screening of tens of thousands of maize plants, a regular supply of hundreds of thousands of first-instar larvae is required. Attempts were made over several years and at several research institutes to artificially rear *B. fusca* on meridic diets, but to no avail. The problem was the almost negligible survival of first-instar larvae on the various diets. Only after developing to the second instar (either on meridic diet or on growing maize plants) did larvae successfully complete development on meridic diets to the pupal stage. Attempts at laboratory rearing were therefore terminated.

The current method of providing first-instar larvae for field infestations involves collecting diapausing larvae during late winter (late July to early September) from maize stalks in fields that were planted specifically as a trap crop very late in the season, in early January. These plants are at a very attractive stage (knee height) in early February when the second-generation moth population is active. Extensive oviposition occurs, resulting in a 100% larval infestation. At the pre-pupal stage, larvae bore into the stems to overwinter, and it is in the diapause state that they are collected several months later.

The maize stalks are dug out and stacked in piles to await manual extraction of the larvae by field workers. The stems are split open using a sturdy knife, and the larvae are carefully tipped out onto a sack covering the worker's legs. The larvae are then scooped up with a plastic spoon and placed into 5-liter cardboard waxed ice-cream containers quarter-filled with 1- to 2-year-old pine wood shavings. The shavings are first sieved to remove pieces larger than 10 x 10 mm, and then sieved again to remove fine sawdust particles which result in high larval mortality if left in the containers. The spoon is used to avoid larvae being squeezed by workers picking them up manually. It was found that manual handling caused substantial larval death, and that using a spoon reduced larval mortality considerably. It was also observed that larval mortality increased when the containers were completely filled, due presumably to the tightly packed shavings either puncturing or bruising the skin during larval movement.

Larvae are transferred in the field periodically during the day from the 5-liter containers into 100- x 15-mm clear plastic petri dishes, which are half-filled with wood shavings similar to those used in the 5-liter containers. Ten larvae are placed into each petri dish, and these are stacked and stored in a conventional seed store cold room (unlit, 7° to 10°C) for several months until the larvae are required.

As the host plant resistance program involves the artificial infestation of tens of thousands of plants, the planting and infestation have to be spaced out over a 9-week period. It is essential, therefore, that not all the larvae emerge from the diapause state simultaneously, but that controlled pupation, hence moth emergence, oviposition, and supply of first-instar larvae, should occur. Diapause larvae can be stored for up to 5 months under cold room conditions. In the spring, the larvae are brought out of the cold room into the laboratory where the temperature is controlled at 31° to 34°C (day) and 19° to 23°C (night) and the light regime is 15:9 h light:dark. The larvae come out of diapause 30 to 50 days later, depending on how long they have been in the cold store. The longer they have been cold stored the longer they take to emerge from the diapause state and pupate. Larvae collected in early July and placed immediately in the laboratory take about 50 days for pupation to commence, while larvae collected at the same time and cold stored for 40 days take about 80 days to commence pupation. Larvae

Table 1. Percentage of all first-generation egg masses laid on maize plants (n = 100) of different heights (measured from ground level to the funnel top)

	Plant height (cm)						
	0-25	26-35	36-45	46-55	56-65	66-75	76-85
%:	0	45	32	15	6	2	0

Table 2. Number of eggs per egg batch laid by first-generation moths expressed for each batch as a percentage of the total number of egg batches (n = 176 egg batches)

	Number of eggs/egg batch								
	1-5	6-10	11-15	16-20	21-25	26-30	31-35	36-40	
%:	1	9	31	24	24	6	3	2	= 100%

collected in late August and placed immediately in the laboratory take only about 24 days for pupation to commence, while larvae cold stored for 40 days take 50 days to pupate.

Pupation extends over a period of at least 12 weeks after larvae are brought into the laboratory. The pupal stage lasts for 20 to 22 days at laboratory temperatures. The petri dishes are checked for moths every 20 days, when all pupae and dead larvae are removed. Pupae are placed 200 per 5-liter cardboard waxed ice-cream container, and these containers are checked daily for moth emergence.

Unsexed moths are placed 20 per 5-liter container, and supplied with small cylindrical glass bottles (26 x 80 mm) wrapped spirally with household waxed paper as an oviposition substrate, and a piece of maize leaf approximately 10 x 20 cm as an oviposition stimulus. The presence of the leaf results in a marked increase in egg laying. These glass bottles are removed and replaced daily. All egg masses laid on the waxed paper are stripped off by placing the paper on a table top and running a blunt knife between the paper and the eggs. To facilitate handling and weighing, the eggs are separated by washing with tap water. The egg masses collected each day are placed on laboratory paper toweling fitted into a large glass laboratory funnel, and sprayed with water. The eggs separate readily, and the paper is then laid flat for the eggs to dry.

After a few hours, the eggs are brushed off and separated into 600-mg lots. The eggs are placed in small open glass bottles (10 x 50 mm, each containing 600 mg of eggs). The bottles are kept at 31° to 34°C (day) and 19° to 23°C (night) in 5-liter cardboard containers supplied with a small piece of water-soaked cotton to maintain high relative humidity. When the eggs reach the black head stage (5 to 6 days), the bottles are plugged with cotton stoppers to prevent larval migration after eclosion.

Within one day after larval eclosion, 600 mg of the larvae plus egg shells are thoroughly mixed with 100 g of maize meal (sifted to remove the very fine powdered maize meal that causes high larval mortality) by pouring the mixture back and forth several times through a glass funnel (plastic funnels are avoided as they set up static) into 1-liter glass laboratory beakers. After about 20 such mixings, a mechanical applicator is filled with the mixture and the calibration is checked. The pre-determined mass of 600 mg of larvae (containing approximately 6,000 larvae) plus 100 g of maize meal ensures that 2 doses (0.33 g of mixture/plant) delivered by the applicator into each plant funnel introduce between 16 and 22 larvae/plant if the larvae and maize meal have been thoroughly mixed. The calibration consists of delivering two doses into each of ten glass petri dishes, and then counting the larvae in each dish. Once the mean of 10 petri dishes is about 20 ($\pm$ 3) larvae per petri dish, field infestation commences.

Methods of Infestation

All maize plants are infested when they reach a height of about 35 cm with two doses each of 8 to 11 first-instar larvae, giving a total of 16 to 22 first-instar larvae per plant. More than this number of larvae often results in such severe damage to the developing tassel that pollination is not possible, or to the stem tissue so that no grain develops. Less than this number of larvae results in too many apparently "resistant" plants surviving. Attempts to introduce black head stage eggs into the plants instead of first-instar larvae were unsuccessful, presumably due to the effect of low relative humidity on larval eclosion.

In the maize breeding program plants are spaced 45 cm apart in the row, with 10 plants normally planted per row, and rows planted 90 cm apart, giving a population of 25,000/ha. Occasionally seeds are planted every 22.5 cm in order to get 20 plants per row. Depending on the material to be infested, either 6/10, 10/10 or 20/20 plants are infested. Where inbreds are screened for resistance, only 6 out of 10 plants are infested, and the remaining 4 plants are used for stunting comparisons, and for seed at harvest in case the infested plants are so badly damaged that they yield no grain. Where segregating material (S_0 to S_3) is planted, all the plants in the row (normally 10) are infested so that the more resistant plants can be selected at harvest. Where populations or composites are to be screened for the first time, 20/20 plants in a single row are infested, and an impression is gained of the level of resistance present in each population; those populations that show a higher than average level of resistance are then planted out in greater quantities the following season, and development of resistant germplasm is begun.

Damage Evaluation

As mentioned above, larvae feed on several different parts of the maize plant, and to attempt to obtain resistance in each of these feeding

Figure 1. Leaf damage after 25 days feeding by *B. fusca* in resistant maize ("1" rating).

ites would be an improbable goal. ecause larvae do most of their eeding in the whorl tissue, attempts identify sources of resistance have entered around this feeding site.

t was noted by Barrow (1985) that ating leaf damage after 21 to 25 ays feeding on a scale of 1 = very ittle damage to 5 = severe leaf hredding is a quick and efficient ield method of first generation lamage assessment (Figures 1 and).

Any feeding period shorter than 21 days does not allow sufficient time or severe damage to occur, and ating whorl damage any later than 25 days can often run into problems vith tassel emergence in early ultivars. With this rating system nly plants rated 1 to 3 are selected t harvest. Damage ratings for nbred lines are taken on each of 6 nfested plants out of the row of 10 plants. Uninfested control plants are available for stunting comparisons. A stunting rating on a scale of 1 to 5 is taken in addition to a leaf damage rating. At harvest the extent of stunting of the stem between the assel and ear of each plant and also of the ear size is considered in making selections.

For segregating material, all plants in the row are infested and rated individually, self-pollinated, and the better ones selected at harvest. Although assessment of stem feeding is an important part of resistance breeding, no routine splitting of stems takes place. It is far too laborious, and the size of ear is often an indication of stem tunneling. Earlier work has shown that yield loss in several inbreds was significantly correlated with the amount of stem boring ($r = +0.56$, $p < 0.01$) (Barrow, in press). Plants that showed severe stem boring also showed significant reductions in plant height ($r = +0.73$, $p < 0.01$). It was concluded that field selection for resistance to *B. fusca* should rely on leaf damage recorded after $\pm$ 24 days feeding and visual assessment at harvest of plant height and yield reduction.

Sources of Resistance

No maize germplasm has yet been identified as showing immunity to *B. fusca*, but a wide range has shown intermediate resistance to first-generation (whorl feeding) larvae. These sources include locally adapted inbred lines and populations, as well as exotic material from the U.S. Corn Belt.

gure 2. Leaf damage after 25 days feeding by *B. fusca* in isceptible maize ("5" rating).

The resistance has been sufficient to retard larval growth or to result in the death of varying percentages of the larvae feeding in the whorl of these plants. No research has yet been carried out on resistance to the second-generation stalk borer, which occurs in late summer and damages the developing ear and stem.

Measuring the Effectiveness of Resistance

The effect on *B. fusca*

Infestation of several local and exotic inbreds by Barrow (1985) showed that the mean number of *Busseola* larvae feeding in the whorl tissue of these inbreds ranged from 1.08 to 4.57 larvae/plant after 15 days feeding, and the mean larval biomass/plant ranged from 44.6 mg for the most resistant inbred up to 648.2 mg for the most susceptible inbred. A highly significant correlation ($r = 0.65$, $p < 0.01$) was apparent between larval biomass recorded after 15 days feeding and leaf damage ratings taken after 21 days feeding. This variation in biomass was ascribed to two resistance factors: the first is thought to be a short-lived, but effective resistance factor in the whorl tissue which either kills or repels early instar larvae, resulting in fewer larvae feeding in those plants, while the second, operative for most of the larval feeding period in the whorl, retards development and hence weight gain of larvae. Unpublished data have shown that there are two distinct mechanisms affecting larval numbers—one is repellent, and the other antibiotic.

The effect on the maize plant

For resistance to be effective, there must be minimal loss in crop yield under borer infestation. If the farmer loses more than the cost involved in chemically controlling the pest, then resistance is of no value to him, and he may as well control the pest chemically at a lower cost. In an investigation of yield loss of several inbred lines under artificial infestation, Barrow (in press) showed that yield potential of the genotypes varied significantly under borer attack, yield reductions ranged from 38% in the least susceptible inbreds

to 100% in the most susceptible. In 10 single-cross hybrids, Barrow (unpublished) showed that yield losses ranged from 15.8 to 46.3%. The inbreds, being smaller plants, sustained proportionately far greater stem damage and showed far greater yield losses than the hybrids did. The correlation coefficients for the hybrids for yield and both leaf and stem damage were $r = 0.36$ ($p < 0.05$) and $r = 0.56$ ($p < 0.01$), respectively, and for the inbreds were $r = 0.39$ ($p < 0.01$) and $r = 0.56$ ($p < 0.01$), respectively.

As the hybrids in these yield trials contained the least susceptible elite inbred lines in the Pioneer breeding program, and not inbreds bred specifically for resistance to *Busseola*, it is probable that hybrids made up with resistant lines will show lower yield losses. However, to be of commercial use, the hybrids must also be high yielding. Several yield trials with such hybrids are in progress during the 1986/87 season.

Development and Utilization of Resistance

The ultimate objective in a commercial company's host plant resistance program is to release a stalk borer-resistant commercial hybrid. No analysis has yet been carried out to determine the nature of resistance nor its inheritance, but from preliminary experiments inheritance appears to be additive. No major dominant genes for resistance have been identified. The major thrust of research has been in the development of borer-resistant populations and inbred lines. It is hoped that when populations or inbreds have been developed with a reasonable level of resistance, the germplasm can be incorporated into hybrids, making them less susceptible to stalk borers than present-day hybrids. Attempts to recover elite material with resistance to stalk borer by a backcross recovery program have failed due to the polygenic nature of resistance inheritance. At present two methods are used to develop resistance to *B. fusca* (Figure 3).

Population development

Full-sib recurrent recombination under infestation is used primarily to develop resistant populations that can be used as female parents in topcross hybrids or, after at least 3 years of full sibbing under infestation, as sources of resistance for the development of inbred lines.

For borer-resistant population development, 50 to 100 unscreened local and exotic populations are planted at a high population of 20 plants per 4.5-m row, and infested with larvae. Individual plants are rated after about 25 days feeding, and those populations that show better resistance than others are planted out the following year (year

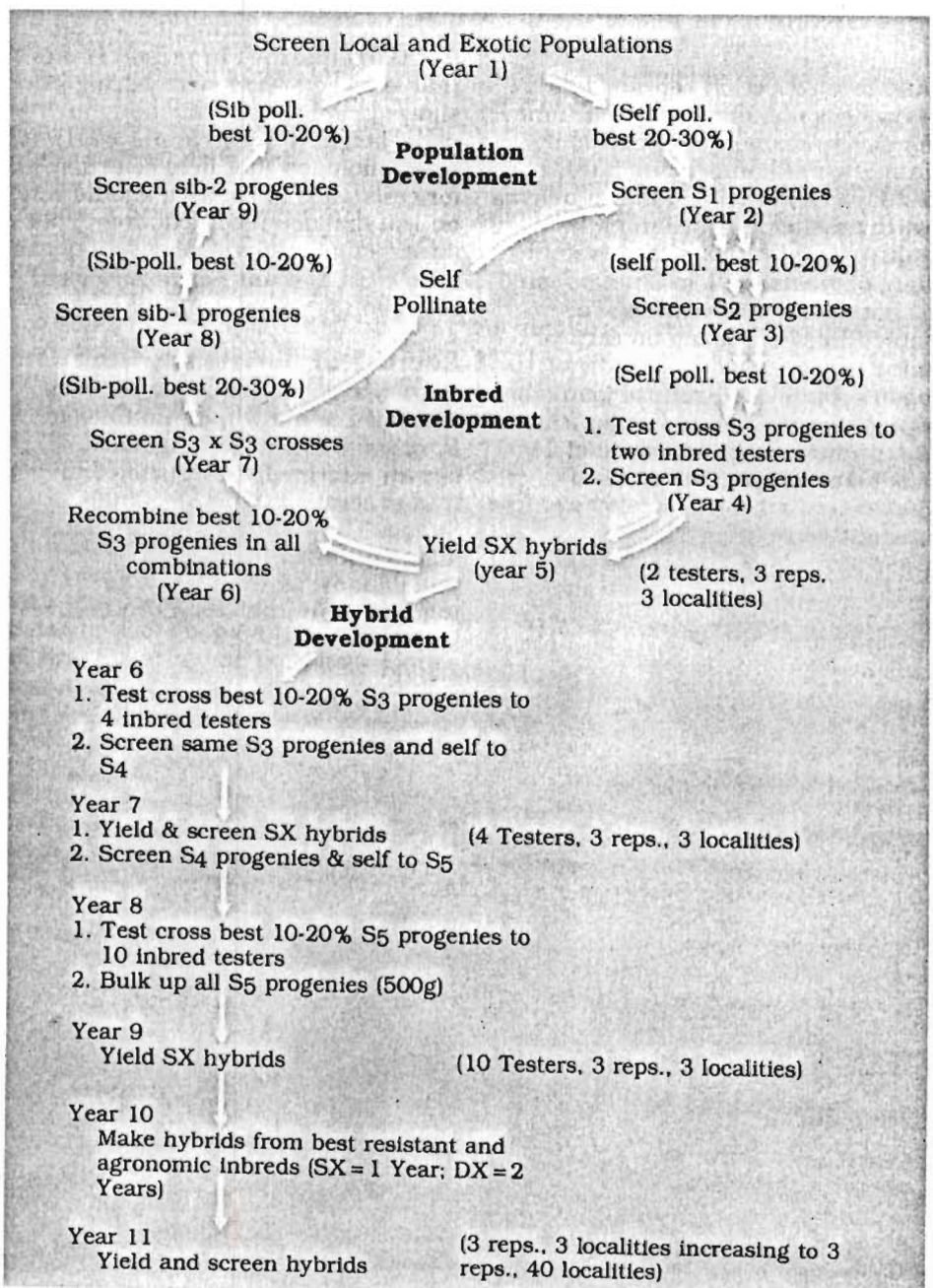


Figure 3. Development of borer-resistant populations, inbred lines, and hybrids.

1) in larger numbers, normally about 200 to 300 plants. These plants are infested, rated, and the worst 70 to 80% are removed soon after rating. The remaining 20 to 30% are self-pollinated and selection at harvest is based on the leaf damage rating, stunting of the plant (especially the part between ear and tassel), and ear size.

The S_1 progenies are planted out ear to row the following season (year 2), infested, and the best 10 to 20% self-pollinated. The best ears are selected and the process repeated for year 3. In the fourth year, the S_3 progenies are screened again to obtain another season's rating, and are also testcrossed to 2 inbred tester parents, and these single-cross hybrids are yield tested in year 5 at three locations.

Once the agronomic data are available, several of the best resistant and agronomic S_3 selections in each year's program are crossed with each other (year 6) in as many combinations as are practical. The following year (year 7) the crosses are planted out in two fields — one where the crosses are self-pollinated for pedigree breeding of inbred lines, and the other where the crosses are recombined under infestation by sibbing the best 20 to 30% of the plants. In year 8, the sib-1 progenies are screened again under artificial infestation, and the best 10 to 20% of the plants are sibbed again. This process is repeated in year 9, and in year 10, corresponding to year 1, selfing of material starts the cycle again.

Inbred development

Pedigree breeding is utilized by recombining the most resistant S_3 selections in single crosses, followed by inbreeding under infestation to the S_3 stage. All S_3 progenies (including those submitted by other breeders at Pioneer) are testcrossed to 2 testers (year 4), and the hybrids are yield tested at three locations the following year. Using the yield data and resistance ratings of the initial S_3 progenies, 10 to 20% of the best S_3 progenies are recombined in year 6 in a full-sib program for population development as described above, and are also individually crossed to 4 tester parents for yield trials at three locations the following season. In addition, the lines are screened again under borer infestation and selfed to the S_4.

The following season, year 7, the single-cross hybrids are yield tested and screened, and the lines are also screened again and selfed to the S_5. From those results, the best 10 to 20% are testcrossed the following season to 10 tester parents to be similarly yield tested the following season and the lines selfed and bulked. The resultant single-cross hybrids are yield tested in year 9 and from the results, hybrid predictions are made. The potential commercial hybrids are made up the following season, to be yield tested a year later. At all times in line selection, detailed notes are taken on agronomic as well as resistance characteristics, as any new hybrid will have to be competitive against commercial (susceptible) hybrids.

It is therefore important that any inbred lines developed with resistance to *B. fusca* be competitive when yield tested in single-cross combinations with other susceptible, but advanced, inbreds. Selection of plants in the *Busseola* program is based primarily on resistance, but it is essential that the resistant plants are agronomically sound as well. This unfortunately limits the selection of resistant material, but by introgressing resistant material into locally adapted populations or by crossing less adapted but resistant inbreds with elite inbreds, and inbreeding directly from these single crosses, good progress has been made in developing agronomically sound and less susceptible inbreds.

In a recent screening of 296 inbreds submitted by the author and several Pioneer breeders, the 24 borer-resistant selections submitted were rated as follows: 2 had excellent resistance (rated 1), 12 had good resistance (rated 2), and 10 had low resistance (rated 3). Of the other inbreds screened, 3 had good resistance (rated 2), 12 had low resistance (rated 3), and 257 were susceptible or very susceptible (rated 4 or 5). Several double-cross hybrids involving 1, 2, 3, or 4 resistant inbreds are currently being evaluated for resistance to whorl feeding *B. fusca*.

Usefulness in Pest Management

Present methods of stalk borer control include either the application of a granular systemic carbamate in the furrow at planting, granular insecticides (carbaryl, endosulfan and trichlorton) applied into the whorl by tractor or hand, or tractor/aerial application of liquid insecticides. The carbamate is about 10 to 15 times more expensive than the whorl-applied granules and sprays, but is often used because it also gives good control of other soil insects as well as leafhopper vectors of maize streak virus.

However, the majority of maize farmers rely on whorl-applied chemicals, of which liquids predominate. The general recommendation for whorl-applied granules is to apply them about 10 to 14 days after the commencement of damage, as the larvae are still small enough to not have bored deeply into the funnel or stem, and the funnel has not been damaged to the extent that granular insecticides will not get to the larval feeding site. It is important to apply the granules only after damage has commenced, as the chemicals mentioned above are only active for 3 to 4 days, and have no preventative value. Contact liquid chemicals are applied both preventatively and curatively, but are generally ineffective once the larvae are about 10 mm and larger and are feeding well within the whorl leaves. Several preventative sprays are therefore necessary.

Systemic insecticides such as monocrotophos are effective against large larvae, but when applied by tractor or aerially, have a severely detrimental effect on parasites and predators. The usefulness of borer

resistance in maize therefore has to be viewed in the context of the above control methods.

If totally resistant hybrids were available, obvious financial benefit would accrue to the farmer. But the resistance appears to be additive and the great majority of commercial hybrids are 3-way or double crosses (4 parents). The chances of obtaining three or four resistant and agronomically superior inbreds are remote. The best situation would probably be hybrids with one or two resistant inbred parents conferring partial resistance or lowered susceptibility to *B. fusca*.

Correspondence with several researchers has indicated that partial resistance is a desirable trait to incorporate into hybrids (D. Barry; W. Guthrie; B. Wiseman; A. Hallauer; W.P. Williams, personal communication). If the yields of such hybrids were appreciably below those of other commercial hybrids, farmers would probably elect to plant the higher yielding susceptible hybrids, and chemically treat any borer infestation which developed. If these hybrids were as high or nearly as high yielding as other commercial hybrids, farmers would then definitely favor the less susceptible hybrids, as either control costs would be lowered, or damage would be subthreshold. In addition, irrespective of the level of infestation, many farmers do not apply control measures at all, particularly in the low potential areas. Planting a resistant or semi-resistant hybrid should result in higher yields.

The three borer resistance factors that have been identified so far have acted either singly or in combination to reduce the larval biomass and feeding on the plant, resulting in less damage and less yield loss. The fast acting factor that kills first-instar larvae gives an immediate benefit in that larval populations are substantially reduced in number, hence causing less damage, possibly even a subthreshold level that requires no control. The second factor, which repels larvae, also results in fewer larvae feeding on the plants, as well as in larvae migrating from plant to plant, thus exposing them to adverse environmental conditions, predators, and parasites. Ampofo (1986) mentions that any prolongation of larval dispersal of *Chilo partellus* exposes the larvae to these mortality factors, and could be exploited in a resistance development program.

The third factor, which causes slower larval development on resistant germplasm than on susceptible germplasm, results in the larvae feeding for longer periods on the plant. This retards damage development and allows more time for chemical control. It is also possible that by delaying the development of first-generation larvae, the second generation may be extended to the extent that the larvae are not fully prepared to enter diapause at the onset of autumn, and a proportion of these larvae could be killed by winter frosts, thus reducing the infestation level the following spring.

References

Ampofo, J.K.O. 1986. Effect of resistant maize cultivars on larval dispersal and establishment of *Chilo partellus* (Lepidoptera: Pyralidae). Insect Science and Its Application 7(1):103-106.

Barrow, M.R. 1985. The effect of different maize genotypes on the maize stalkborer, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae), feeding in whorl tissue. The Journal of the Entomological Society of Southern Africa 48(1):113-119.

Barrow, M.R. The effect of the first generation maize stalkborer, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae), on yields of different maize genotypes. The Journal of the Entomological Society of Southern Africa 50(2). (In press.)

Du Plessis, C. and H.A.P. Lea. 1943. The maize stalk-borer, *Calamistis fusca* (HMPS). Bulletin of the Department of Agriculture, Union of South Africa. No. 238.

Egwuatu, R.I. and C.B. Ita. 1982. Some effects of single and split applications of carbofuran on the incidence of and damage by *Locris maculata, Busseola fusca* and *Sesamia calamistis* on maize. Tropical Pest Management 28(3):277-283.

Kaufmann, T. 1983a. Observations on the host plant adaptation of *Busseola fusca* (Lep: Noctuidae) in Nigeria. Proceedings of the Entomological Society of Washington 85(2):321-326.

Kaufmann, T. 1983b. Behavioural biology, feeding habits, and ecology of the three species of maize stem-borers: *Eldana saccharina* (Lepidoptera: Pyralidae), *Scsamia calamistis* and *Busscola fusca* (Noctuidae) in Ibadan, Nigeria, West Africa. The Journal of the Georgia Entomological Society 18(2), April:255-259.

Kuhn, H.C. 1978. Selection for resistance against the maize stalk-borer (*Busseola fusca*). Proceedings of the 3rd South African Maize Breeding Symposium. Potchefstroom, 1978. Technical Communication of the Department of Agricultural and Technical Services of the Republic of South Africa No. 152:84-86.

Mlambo, S.S. 1983. The status of cereal stem-borer and legume pod-borer research in Zimbabwe. Insect Science and Its Application 4(1/2):221-222.

Ogunwolu, E.O., K. Nwosu and S.O. Ogunyebi. 1981. Stem borer damage in maize as affected by host plant density. The Journal of Agricultural Science, Cambridge 96:695-697.

Revington, J., J.B.J. van Rensburg, G. Burghardt and W. Knauf. 1984. Preliminary field trials, with a pheromone based monitoring system for the maize stalkborer, *Busseola fusca* (Fuller) (Lep: Noctuidae). The Journal of the Entomological Society of Southern Africa 47(1):107-113.

Seshu Reddy, K.V. 1985. Relative susceptibility and resistance of some sorghum lines to stem-borers in western Kenya. Insect Science and Its Application 6(3):401-404.

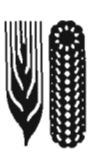
Usua, E.J. 1968. The biology and ecology of *Busseola fusca* and *Sesamia* species in South-Western Nigeria. I. Distribution and population studies. The Journal of Economic Entomology 61(3):830-833.

van Rensburg, J.B.J. 1981. Die plaagstatus van *Busseola fusca* (Fuller): 'n studie van plaag-tot-gewasverhouding. Ph.D. thesis. University of Stellenbosch, Stellenbosch, South Africa.

Walker, P.T. 1981. The relation between infestation by Lepidopterous stem borers and yield in maize: methods and results. EPPO Bulletin 11(2):101-106.

Walters, M.C. 1974. Mechanisms of resistance to insects and application to maize stalk-borer. Proceedings of the 1st South African Maize Breeding Symposium. Potchefstroom. Technical Communication of The Department of Agricultural and Technical Services of the Republic of South Africa No. 132:83-87.

ISBN 968-6127-35-6



Centro Internacional de Mejoramiento de Maíz y Trigo
International Maize and Wheat Improvement Center
Lisboa 27, Apartado Postal 6-641, 06600 México, D.F., México