ANTIXENOSIS AND ANTIBIOSIS AS RESISTANCE MECHANISMS OF SOUTH AFRICAN SUGARCANE VARIETIES AGAINST EARLY INSTAR LARVAE OF *ELDANA SACCHARINA* WALKER (LEPIDOPTERA: PYRALIDAE)

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ABSTRACT

The complexity of the behaviour of neonate *Eldana saccharina* Walker (Lepidoptera: Pyralidae) larvae and the limited information on their response to the morphological characteristics of South African sugarcane varieties was the primary justification to study antixenotic/antibiotic effects on larval behaviour. Laboratory experiments were conducted with stalk segments in plastic jars inoculated with larvae and in a metal cage covered with gauze. In jars, the larvae were observed until they penetrated the stalks. After 14 days, the stalks were dissected and larvae weighed. In all varieties, larvae moved directly to the node after inoculation and penetrated the stalk through leaf scars and buds. No significant differences in larval mass were observed among varieties. In cage experiments different parts of the node, namely the rind below the wax band; the bud; and the root primordia were tested. There was a clear indication that rind hardness and the budscale properties are associated with varietal resistance and only affect early instars. The experiments were repeated using whole cane plants in a glasshouse. The results were similar to those of laboratory experiments.

In the Insect Rearing Unit, scraped waxes from different varieties were incorporated into the diet. Larval masses from different diets showed significant differences among varieties, but they did not conform to the known resistance ratings, as cane varieties N12 and N21 showed high susceptibility, instead of resistance.

Dispersal behaviour of neonates shortly after hatching was investigated in 'mobility experiments' conducted on live cane plants. Mobility is important because the more time neonates spend wandering around on the stalk surface or on exposed parts of the plant, the more vulnerable they are to predation and other adverse factors that may

reduce their survival. Experiments to test stalk penetration by larvae on the node showed that neonates required a softer food source before attacking the hard nodal parts. Second and third instar larvae were used subsequently to the mortality of all neonates fed on the rind, which in turn resulted in non-significant differences, suggesting that feeding on debris and/or leaves is critical to the survival and penetration of larvae into the sugarcane stalk.

Incorporation of the characteristics tested in these experiments aims to reduce the number of larvae that penetrate the stalk and to expose them for longer on the surface where their numbers may be controlled by predators and insecticides. The resistant varieties used in these experiments have high fibre and less sugar, but newer varieties, such as N29 and N33 incorporate both high resistance and high sucrose yield, which are the two key elements for optimised sugar production. Chemical characteristics of the plants need to be taken into consideration as high sucrose is seldom found in fibrous varieties. Leaf sheath tightness is another characteristic that would go well with leaf sheath hairiness, because though not tested in this work—would make it difficult for the larvae to get to the smooth adaxial surface of the leaf. The hardness of trichomes is another feature that needs to be investigated, because a variety may have dense, but soft pubescence that does not repel even the most sensitive larvae, neonates. At present, integrating plant resistance with cultural control, i.e. field hygiene etc. is cost-beneficial for the sugar industry.

Keywords: Antixenosis, antibiosis, *Eldana saccharina*, sugarcane, larval behaviour.

PREFACE

This study represents the original work of the author and has not been submitted in any form to another university. Where the author used the work of others, it has been duly acknowledged in the text.

L. Y. Mabulu

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CHAPTER 1: INTRODUCTION

The stalk borer, *Eldana saccharina* Walker is indigenous to Africa and surrounding islands, and has been known as a pest of agricultural crops for about 100 years (Carnegie, 1983). It is a cryptic insect, which at the immature stages of development in the sugarcane habitat, is well protected from both natural and applied controlling factors. It was a pest in the early 1940's, but with the advent of harder cane varieties, it disappeared until 1970 when a heavy infestation was recorded in a field with cane variety NCo376 at Hluhluwe, as well as Pongola, Mpumalanga, Swaziland and low altitude areas of KwaZulu-Natal south coast (Carnegie, 1974). Any of the South African sugarcane varieties may be affected. *Eldana saccharina* is endemic in more than 80% of the sugarcane region and is absent only in high altitude areas (Nuss *et al.*, 1986).

Previous host records of *E. saccharina*, summarized by Girling (1972) have tended to stress the crop or gramineous hosts, but *E. saccharina* probably evolved on the Cyperaceae. *E. saccharina* can colonise a variety of different crops such as cassava, millet, maize, sorghum, rice and bullrush millet and wild plants such as pigweed, elephant grass, guinea fowl grass and wild sorghum (Girling, 1972). The initial invasion of sugarcane by *E. saccharina* from neighbouring *Cyperus papyrus* swamps in northern KwaZulu-Natal (Atkinson *et al.*, 1981) may have been facilitated by flavonoid similarities between these plant families (Rutherford, 1998). Atkinson (1979) tested female moths for their egg-laying preferences between a natural host (*C. immensus*), a crop host (sugarcane) and a non-host (*C. latifolius*). A dicotyledon, *Amaranthus spinosus* L. was also included. The results of three insectary choice trials showed that *E. saccharina* preferred dead material, green tissue rarely being used (Atkinson, 1979; Leslie, 1990; Mabulu & Keeping, 1999). No eggs were found in the flowers of *C. immensus*, possibly for this reason. Although not the preferred host, sugarcane appeared to have been actively chosen, perhaps because it has abundant dead leaf material around its base. For example, the oviposition frequency in sugarcane was twice

that in the sporadically utilised host *C. latifolius*, and twice that in random, inappropriate sites (which would not occur in nature) (Atkinson, 1979).

Feeding sites in the Cyperaceae are nearly always in the rhizome. In *Papyrus* spp, where the rhizome is usually submerged, the common feeding site is in the inflorescence, but when rhizomes are exposed by falling water levels, they become favoured feeding sites and high larval intensities may occur (Atkinson, 1978b). In *C. immensus* the inflorescence is favoured during flowering (September to February) when up to 90% of the population is found in the inflorescence, particularly from October to December when peak population intensities occur in this host. The young larvae presumably migrated to the inflorescence after hatching, because larvae were sometimes encountered feeding on the leaves of *C. immensus*, having dispersed from their hatching sites. Two Cyperaceae, *Cladium mariscus* and *Cyperus latifolius*, are attacked in the inflorescence only, while in *Kyllinga* spp. *E. saccharina* feeds on all parts of the plant (Atkinson, 1979).

In crop hosts, *E. saccharina* generally attacks older plants. Younger crop hosts are only attacked in severe outbreaks. In sugarcane in southern Africa, this borer attacks the lower third of mature stalk. In cases where this borer has been found in maize, it has also generally been found in the lower third of the mature stalk. Surveys for natural enemies of *E. saccharina* in east and West Africa showed differences in behaviour of this borer. Borings were found more in the top third of sugarcane stalks than in the bottom third. NCo376, a South African variety found at a Kenyan sugarcane estate, was surveyed and the damage though not marked at the top of the stalks, was much higher than in the bottom third of the stalk. The same variety at the same age was also surveyed in South Africa in the same way as in Kenya with different results. This shows that there is a difference in boring behaviour between the higher altitude Kenyan and Ugandan populations of *E. saccharina* when compared to the coastal population found in South Africa (Conlong, 2000).

Within each segment, feeding usually starts around the node; this is the most common penetration site, and extends into the internode or throughout the segment(s). Beyond reflecting the

penetration pattern, there was no evidence that feeding tended to concentrate around the node (Atkinson, 1979).

Cracking of cane undoubtedly aids penetration (Atkinson, 1978b) and the low proportion of penetration through cracks probably reflects the incidence of cracking in the field, rather than the exploitation of cracks by *E. saccharina*. The internode is rarely penetrated unless cracked. Once cane has become severely damaged by borings, there is evidence in the field that succeeding generations often penetrate through old borings. The length of cane damaged by individual larvae is very variable, but from 2 to 8 cm of feeding is required to produce a mature individual (Atkinson, 1979).

The nitrogen level in the unsubmerged rhizome of papyrus is remarkably high, and this suggests why this feeding site is so favoured by *E. saccharina*. *Papyrus* inflorescences are also relatively nutritious, but the inflorescences of *C. immensus*, sampled in February 1979, were senescing and may not reflect the optimum/maximum nitrogen levels of this feeding site. The sugarcane stalk is apparently not a particularly nutritious material compared with others utilised by *E. saccharina*, especially unsubmerged papyrus rhizome; and available figures show a small difference between cane and the most common wild host, *C. immensus* (Atkinson, 1979).

The coast between Richards Bay and the Umvoti River mouth represents a region of marked ecological change. The Mozambique coastal plain, which extends into northern KwaZulu-Natal with its associated lakes and marshes, ends at the Umlalazi River mouth (Mtunzini). Papyrus extends no further south than this point and Vogel *et al.* (1978) have shown that here the frequency of C_4 grass species falls from 100% to between 75% and 95%. It is within this region of ecological change that sugarcane has (on average) been more heavily infested than elsewhere in the KwaZulu-Natal cane belt. Similar distribution patterns occur further inland. For some reason the various host species are free from attack as conditions become cooler. Until 1980, the distribution of *E. saccharina* in sugarcane in South Africa was restricted to a relatively narrow coastal zone in

KwaZulu-Natal, probably limited by cold winter temperatures (Atkinson, 1980). Subsequently, however, field surveys and light trapping showed that *E. saccharina* had spread into the Midlands. This became particularly apparent in recent drought years. The spread of *E. saccharina* into a region which, in the past, was regarded as too cold to allow normal larval development (Atkinson, 1980), has raised questions about the biology and adaptability of the insect (Price, 1984). For this reason, Way (1994) conducted studies aimed at determining the effect of temperature on the longevity, mating success and fecundity at the range of temperatures to which it may be exposed.

One of the theories proposed to explain the presence of *E. saccharina* in the Midlands is that temperatures have recently increased, making it possible for the insect to be active and to mate at night in a region previously believed to be too cold. This is supported by an upward trend in the winter temperatures in the Midlands, particularly over the last few years when the mean winter temperatures were 98%, 99%, 102% and 106% of the long term mean. In the summer in the Midlands, *E. saccharina* is capable of mating because the mean summer temperatures have remained at around 20^0C. The increasing occurrence of *E. saccharina* in the Midlands is most likely due to warmer winter temperatures in the region, coupled with the insect's ability to reproduce at these temperatures. Another possible theory is that *E. saccharina* has adapted to the lower temperatures by developing lower development thresholds (Way, 1994).

1.1 BIOLOGY OF *E. SACCHARINA*

The stalk borer *E. saccharina* is the larval stage of an indigenous, inconspicuous brown pyralid moth with a wingspan of 30-35 mm, that rests with its wings folded over the back (abdomen). Moths emerge shortly after sunset and mate, and the female begins oviposition after about 24 hours. The female may fly 200 m or more before ovipositing, but usually more eggs are laid closer to the adult's emergence site (Carnegie, 1974). The oviposition sites include the folds of leaf blades and leaf sheaths in the copious dead leaf material produced by sugarcane (Leslie, 1993;

Mabulu & Keeping, 1999). Each egg batch contains about 3-160 eggs (Mabulu & Keeping, 1999) and one female lays about 250 eggs, which hatch after about 8 to 10 days (Leslie, 1986). The hatching larva does not enter the cane stalk immediately, but feeds initially on cane leaves, or else as a scavenger on organic matter (Carnegie, 1974).

After a variable period, the larva is sufficiently robust to enter the plant tissue, and the rest of its immature active life is spent as a borer in the cane stalk. This stalk borer is tough, leathery, brown and very active when removed from its shelter within the stalk (Anon, 1981). Larvae are voracious feeders hollowing out the stalks and pushing frass from them through holes to the exterior (Carnegie, 1974). They may descend from the outside of the stalk on silken threads. They move forward or backwards with equal ease (Anon, 1981). The larval period varies from about 20 days in summer to 60 days in winter, during which time the male larvae moult 5 to 6 times and female larvae 6 to 7 times. There is little published information on the effects of low and high temperatures on the biology of *E. saccharina* (Way, 1994). Dick (1945) found that eggs failed to hatch at 11.1^0C, but did hatch when transferred to 24.4^0C, similar to the results obtained by Way (1994).

The mature larva spins a protective cocoon and pupates within it. The pupa either may be located within the hollowed stalk or be attached to the outside of the stalk, usually beneath a leaf sheath (Carnegie, 1974). The duration of the pupal stage period varies with temperature between seven and 17 days with slight differences between the sexes (Atkinson, 1980). Girling (1978), however, reported very little variation in pupal period with temperature.

Generally, insects respond to increased temperature by speeding up development and activity, which results in decreased longevity and the opposite, occurs at lower temperatures (Chapman, 1969). Longevity and fecundity estimated in the study conducted by (Way, 1994) are within the ranges reported elsewhere. For example, Shanower *et al* (1993) in West Africa studied

E. saccharina reared on artificial diet and reported longevity values of 14.6 and 8.8 days, and fecundity values of 474 and 619 eggs/female at 20^0C and 25^0C, respectively (Way, 1994).

1.2 NATURE OF BORER DAMAGE AND ECONOMIC LOSS

Borer attack on sugarcane may cause any of the following types of damage: germination failures from injured seed-cane, dead tops in older plants, broken stalks, reduced growth of attacked stalks that survive, and loss of cane quality. Both cane and sucrose are thus lost. Borer injury to stalks lessens the amount of juice that can be extracted from them and the percentage of sucrose in this juice; the purity of the juice is correspondingly lowered; total organic non-sugars are increased; and the colour of the clarified juices and syrups is darkened and their turbidity increased. The entrance of fungi and bacteria into the borer tunnels increases this deterioration resulting from borer injury. Borer injury also retards maturation. It is generally considered that bored cane does not keep as well as sound cane after it is cut for milling (Mathes & Charpentier, 1969).

1.3 CHOICE OF VARIETIES

The following criteria are usually put forward in choosing the cultivar of a crop plant to be grown:

1) Suitability for the growing location in question.
2) Efficiency with regard to quantity and quality of the yield.
3) Resistance against abiotic and biotic stress.

For each of the various criteria different significance is attached according to its degree of damage probability. However, grounds of economy or quality may also count in favour of growing susceptible cultivars. This applies to the Bintjie cultivar of potato, which poses an extremely high susceptibility to *Phytophthora infestans* but which will produce a good quality crop and is therefore grown extensively in some regions. In cases like this, very intensive chemical measures must be

undertaken in order to ensure the yield. In many cases, high yield capacity and high resistance to particular strains are combined in one cultivar. Breeding for resistance certainly aims to achieve this combination as far as possible (Heitefuss, 1989).

1.4 THE USE OF RESISTANT VARIETIES

In many ways, the growing of crop varieties that are less attacked than others or yield well in spite of attack is a very good pest control measure (van Emden, 1974). The use of resistant varieties has been recognized for many years as a desirable method of controlling moth borers of sugarcane.

Kogan (1982) listed the following as among the most desirable features of plant resistance from the broader ecological viewpoint:

(1) **Specificity**, plant resistance is usually specific to a pest or complex of pest organisms and seldom has direct detrimental effects on beneficial insects.

(2) **Cumulative effectiveness**, high resistance is not necessary, because the effect on the pest population will be compounded in successive generations.

(3) **Persistence**, most resistant varieties maintain high levels of resistance for a long time, despite the occasional upsurge of biotypes.

(4) **Harmony with the environment**, since no unnatural elements are used, there is no danger of contaminating the environment or endangering humans or wildlife.

(5) **Ease of adoption**, once developed, resistant varieties can easily be incorporated into normal farm operations at little or no extra cost.

(6) **Compatibility**, plant resistance is compatible with other tactics in pest management, being an ideal adjutant when resistance alone cannot maintain a pest below the economic threshold.

1.5 THE DIFFICULTIES OF UTILIZING PEST RESISTANT PLANT VARIETIES

There are also drawbacks to the development and use of resistant varieties. The incorporation of another requirement into the breeding and testing programme greatly increases the work of the plant breeder and difficulty may be encountered in obtaining varieties with the required degree of resistance, particularly when a number of crop varieties or a continual succession of varieties is desirable for agronomic reasons (Mathes & Charpentier, 1969).

In sugarcane, the use of resistant varieties against diseases has achieved great success, but the method has not been prominent in pest control. The reason would seem to lie with the more intimate physiological association of plant and pathogen and the marked resistance or susceptibility so often evident to disease. By contrast, insects tend to be facultative. Resistance approaching immunity to moth borers is not evident among commercial sugarcane varieties (Mathes & Charpentier, 1969).

(a) **Variability between pests**: It commonly happens that resistance to organism A is linked with susceptibility to organism B. Commonly, resistance to pests (often related to a high carbohydrate/nitrogen ratio in the foliage) lowers the defences of the plant to attack by fungal pathogens which are favoured by such ratios (van Emden, 1974). This appears to be true in sugarcane as well, where there is an inverse (significant) correlation between resistance to *E. saccharina* and to smut fungus and mosaic virus across 78 varieties (Heinze *et al.*, 2001).

(b)**'Breakdown' of resistance**: Resistance of a plant is no more a permanent control of an insect pest than is an individual pesticide. Both control measures exert a selection pressure on the pest and the minority strain not affected by the control measure will become more common. The only difference is that this may happen more rapidly with a pesticide (van Emden, 1974).

(c) **Environmental factors**: Plant resistance is the result of an interaction of insect behaviour and physiology with definite plant characteristics. In as much as the resistance characteristics are environmentally variable, so climate or soil type of an area may affect them to render the plant

susceptible. Because of the environmental variability of plant resistance, tests should be carried out in as many seasons and climatically or topographically different areas as possible (van Emden, 1974).

Kogan (1982) noted the following limitations that need to be recognised:

(1) **Time of development**, the method is not adequate for solving sudden or very localised pest problems, because of the long time (3-15 years) necessary to identify sources of resistance and to breed resistant varieties.

(2) **Genetic limitations**, the absence of preadaptive resistance genes among available germplasm may deter use of the method; induced mutations, although possible, would make development programmes longer and more complex.

(3) **Biotypes**, the occurrence of biotypes may limit the use of certain resistant varieties, but plant breeders have been able to avoid this problem by using polygenic resistance or breeding varieties resistant to certain biotypes.

(4) **Conflicting resistance traits**, certain plant characteristics may act as resistance factors for some species, but induce susceptibility to others.

1.6 REASONS FOR THE IMPACT OF INSECT RESISTANT CULTIVARS IN CROP PRODUCTION

(1) **Failure of entomologists and plant breeders to complete their task after identifying the insect resistant germplasm**. Identification of resistance sources is usually and relatively simple. However, incorporating resistance genes into agronomically acceptable cultivars is a much more difficult issue (Teetes, 1985).

(2) **Failure of farmers to accept and use resistant cultivars**. Farmer acceptance and continued use of insect resistant cultivars has been conservative at best. The reasons for

this reluctance are many, partly sociologically based on unfounded, preconceived opinions of the performance of insect resistant cultivars (Teetes, 1985).

(3) **The insecticide support**. Insecticides remain a major control method because they are easy to use, usually effective, and economical and have rapid curative action. In theory, their use in integrated pest management (IPM) strategies demands selectivity by chemistry or application procedure based on real need judged by the use of economic threshold levels. Insecticides provide an escape from the 'pressure' to develop and use insect resistant cultivars because they provide an easy alternative (Teetes, 1985).

(4) **Tendency to separate crop production and crop protection**. IPM has tended to weaken the barrier that has so long existed among agricultural disciplines. In relation to the use of resistant cultivars, and understanding of the role, function and performance of resistant varieties is much more likely achieved as crop production and crop protection specialists unite their objective of producing the most, more consistently, at less expense (Teetes, 1985).

(5) **Failure to produce adequate information about the pest and the resistant cultivar**. Reluctance to change or adopt a new strategy certainly results from inadequate knowledge or assurance that the new approach will succeed. Discovery of a procedure that dramatically and spectacularly controls an insect pest is rapidly and readily accepted. However, most entomologists will accept the fact that in a large majority of cases the insect resistant cultivars that are developed have slight effects on insect pests. In deployment, this is a disadvantage, but the ecological advantages are real. Consequently, plant resistance to insects has unique applicability and function in IPM (Teetes, 1985).

1.7 MOTIVATION

Knowledge of the importance of antixenosis (non-preference) acting on neonate larvae of *E. saccharina* during their initial period on the external surface of the cane plant, and how it affects their survival and success in penetrating the stalk, may assist in the selection of varieties with stalk surface or foliage features that reduce survival and stalk penetration of neonates. If antixenosis proves to be a major mechanism and if features that reduce the ability of larvae to establish on and bore into the stalk can be selected, it would reduce stalk damage from the outset and increase the effectiveness of other control measures acting on neonates, especially predators and insecticides. Also, if we know that larval antixenosis is a major mechanism, we can design screening trials that specifically test larval performance on those plant parts that are apparently important during larval attempts to establish and penetrate.

1.8 OBJECTIVES

The objective of this study was to investigate the role of larval antixenosis in conferring resistance of sugarcane varieties to *E. saccharina*, and especially, to investigate the following:

1. Are there significant differences between varieties (susceptible and resistant) in the time spent by neonates foraging on the surface of the plant before boring into the stalk? Larvae are predicted to have greater mobility (less inclined to establish) on varieties with high antixenotic resistance, which would lead to their spending more time on the plant surface and therefore more time exposed to predators and adverse environmental factors. It would also provide more opportunity for treatment with insecticides. Movement off the plant altogether (into the trash blanket or onto the soil) is predicted to reduce survival even further.

2. What physical and/or chemical plant surface features e.g. budscale, rind hardness, epicuticular waxes, leaf sheath hairiness are associated with diminished or improved ability of larvae to survive, establish, and bore into the plant? Experiments to address this question included

tests of the viability of assays using small sections of intact peri-nodal stalk material, as a screening technique.

(i) **Screening for resistance**: if screening experiments show that neonates target specific parts of the stalk for penetration, then it will be easier to design experiments to concentrate on those specific parts. Screening for resistance was done using stalk segments with the node and segments without nodes. This will determine whether larvae still penetrate the stalk even when they are not given a choice, and through which parts they penetrate. If it is known what characteristics of the various plant parts are unfavourable to the larvae, then breeding for those characteristics can be considered.

(ii) **Budscale hardness**: budscales differ between varieties (Rutherford, 1998). These experiments were a test to see if the budscale played any part in bud/node penetration. Therefore, if penetration by neonates through the buds differs amongst varieties, and also if the buds of susceptible varieties are penetrated more than those of resistant varieties, then budscale physical or chemical characteristics could be investigated further for their potential use in selecting for resistance. If resistant varieties have budscales with characteristics unfavourable for larval growth, then larvae that feed on them are expected to be smaller than or suffer delayed penetration compared with larvae feeding on susceptible varieties. The longer larvae take to penetrate the stalk, the greater their exposure to predators and insecticides. Experiments were conducted on the stalk segments with: a) the budscale removed ('treatment') and budscale intact ('control'); b) budscale intact ('control') and bud completely removed ('treatment'); c) budscale removed ('control') and bud removed ('treatment').

(iii) **Rind hardness**: It has been noted in previous experiments that neonate larvae of *E. saccharina* only penetrate the internode if it is cracked (Atkinson, 1978b). If the screening experiments that compare the degree/frequency of penetration between the node and the internode agree with this observation, then larvae can be confined to the rind on the node (below the wax

band) to see if they feed more when the rind is removed, than when it is intact (rind on the wax intact). If neonates feeding on the rind do not survive or if their survival is lower than those feeding beneath the wax band (with the rind removed), then the hypothesis that the rind is unfavourable for penetration of neonate larvae is supported. Thereafter, larger instars can be used to see if they are also affected by the presence of the rind and its hardness.

(iv) **Epicuticular waxes**: Waxes can be tested for their antixenotic effect on larvae by adding specific quantities from different varieties into the artificial larval used by the South African Sugar Experiment Station (SASEX) Insect Rearing Unit (Graham & Conlong, 1988). If wax chemistry is associated with degree of resistance among varieties (Rutherford, 1994) then we can expect that incorporating wax from susceptible and resistant varieties into artificial larval diet, will produce different effects on the survival and weight of neonates reared in such assays. Larvae were allowed to feed for exactly the same time. They were left for the time they usually require to develop into third or fourth instar.

CHAPTER 2: LITERATURE REVIEW

2.1 HOST-PLANT RESISTANCE

Many researchers are sure that the best plant protection for the future will be found in plant resistance. Genetics controls all the characteristics of a plant whether agronomic, horticultural, or pest management related. Susceptibility, resistance and immunity factors are involved in almost all phenomena that affect plants. When a plant is bred for a desired characteristic, the chosen characteristic normally will persist for long periods if guarded in a careful breeding program. This pattern of resistance or immunity becomes an automatic control built into the seed and is the simplest and the least expensive. One disadvantage is that it is usually time consuming, but the ultimate cost to the grower will be no greater than the cost of the seed. It is no wonder that resistant plants have been enthusiastically received by growers all over the world (Webster, 1975).

Resistance or immunity to pests and plant diseases is as old as each of the evolving plants. Plant diseases and insect problems associated with economically important plant species have been important for thousands of years. During this period of time selection (artificial and natural) in crop plants has produced highly heterozygous populations with enhanced adaptability to local environments and usually some resistance and immunity to many pests and diseases (Smith, 1972).

2.1.1 CATEGORIES EXPRESSING VARIOUS INTENSITIES OF RESISTANCE

Interactions between insects and plants span a wide range of intensities. In terms of the insect, the interaction varies from plants being completely adequate to completely inadequate hosts. Conversely, in terms of the plant species or cultivar, the fewer insect species associated with it, and/or the lower their abundance and the less effect they exert on a plant, the more resistant the plant appears. Resistance usually is measured by using susceptible cultivars of the same plant species as controls. Only immunity, representing complete inadequacy for insects, is an absolute

term, but it is rarely encountered in plants within a host species. The terms **host plant** and **immune** are mutually exclusive (Horber, 1980).

Painter (1951) used the following scale to classify degrees of decreasing resistance: **Immunity**--an immune cultivar is one that a specific insect will never consume or injure under any known conditions. Thus defined, there are few, if any, cultivars immune to the attack of specific insects known to attack other cultivars of the same plant species. **High resistance** is demonstrated by a cultivar that has qualities that result in a small amount of damage by a specific insect under a given set of conditions. **Low resistance** indicates qualities that cause a cultivar to show less damage or infestation by an insect than the average for the crop considered. **Susceptibility**--a susceptible cultivar shows average or more than average damage by an insect. **High susceptibility**--a cultivar shows susceptibility when more than average damage is caused by a specific insect.

The terms indicate the classes used by most workers in insect resistance, as it is observed in the field, without analysis of the mechanisms involved. **Intermediate resistance** is sometimes spoken of as **moderate resistance,** which may result from one of at least three situations. A cultivar denoted as moderately resistant might consist of phenotypically similar plants, some of which have high and others, low resistance because of differences in physiological characteristics. In contrast, a moderately resistant cultivar may be made up of plants derived from a single clone, which is heterozygous for incompletely dominant genes that confer high resistance when homozygous. Moderately resistant plants also may be homozygous for genes which, under given environmental conditions, produce plants that are moderately injured or infested (Horber, 1980).

2.1.2 MECHANISMS OF PLANT RESISTANCE

Observations of insect-plant interactions reveal a wide range of plant suitability as hosts to insects. Variability in plants in the nature and the intensity of interaction is also reflected in the categories and definitions of resistance described in this chapter. They describe the abilities of certain plants to avoid, repel, retard, restrict, or localise insect infestation and damage, or to tolerate it by fast regrowth and recovery from injury. Classifications of resistance phenomena may express the relative success or failure of an insect species to survive, develop, and reproduce on a plant species; or the classifications may describe the relative damage to the host plants in qualitative or quantitative terms (Horber, 1980).

Snelling (1941) included in plant resistance those characteristics that enable a plant to avoid, tolerate, or recover from attacks of insects under conditions that would more severely injure other plants of the same species. Painter (1951) used a more comprehensive definition than Snelling's, describing a plant's resistance as the relative amount of its heritable qualities that influence the ultimate degree of damage done by the insect. In practical agriculture, resistance represents the ability of a certain variety to produce a larger crop of good quality than would other varieties under the same insect population. Beck's (1965) definition restricts plant resistance to the collective heritable characteristics by which an insect species, race, clone, or individual successfully uses the plant as a host. Beck's definition narrows the spectrum of insect-plant interactions to the successful use by the insect of a host, but it excludes the plant's ability to recover or repair losses after injury occurs.

In most growing crops it may be observed that some individual plants either harbour far fewer pests than others or else show relatively little sign of pest damage. These individuals usually represent a different genetic variety from the remainder of the crop, and this variety is said to show 'resistance' to the insect pest. In addition, when different varieties of the same crop are grown side by side, differences in infestation level may be very marked. Resistance to pest attack is

characterised by the resistant plants having a lower pest population density, or fewer damage symptoms, than the other plants which are termed 'susceptible'. Conversely, there will be some plants that appear to be preferred by the pests and these especially susceptible plants will actually be destroyed by the pests and so will not pass on their disadvantageous genetic material (Hill, 1983).

Resistance can be assessed by these four characteristics: (1) Resistance is heritable and controlled by one or more genes. (2) Resistance is relative and can be measured only by comparison with a susceptible cultivar of the same plant species. (3) Resistance is measurable; that is, its magnitude can be qualitatively determined by analysis of the standard scoring systems, or quantitatively by insect establishment. (4) Resistance is variable and is likely to be modified by the biotic environments (Panda & Khush, 1995).

2.1.2.1 SEMIOCHEMICAL-BASED RESISTANCE

Semiochemicals are chemicals mediating interactions between organisms (Law *et al.*, 1971) either within the same species (pheromones) or from different species (allelochemicals) (Nordlund & Lewis, 1976). A semiochemical may influence interactions involving a number of organisms from several trophic levels. Bark beetles, for example, aggregate on trees using semiochemicals produced by conspecific beetles, the attraction of which is synergised by volatiles released from the tree itself (Byers, 1995). The same compounds may attract other insects utilising the tree for food or oviposition, inhibit the development of fungi or bacteria and may also have a role in plant/plant interrelations. A complex naming system has evolved to classify semiochemicals depending on the benefits or detriments resulting from the interaction (e.g. kairomone, allomone, synomone) (Nordlund & Lewis, 1976).

The study of semiochemicals, and the interactions they mediate, are part of chemical ecology and contribute to an understanding of the behaviour, development and evolution of

organisms. However, from a practical point of view, such research also provides the basis for successful use of semiochemicals for pest control as an alternative to exclusive use of broad-spectrum toxicants. Insects use chemical information from their environment at all stages of development, to locate food, oviposition and hibernation sites, to come together with conspecifics and sexual partners, and to avoid dangerous situations or unsuitable habitats and hosts. Semiochemicals that have the ability to attract or repel insects, or that enhance (synergise) or inhibit the action of other chemicals, have the potential to be used in direct control of pests by mass trapping or mating disruption, or in deterring pests from food and oviposition sites (Silverstein, 1990). Semiochemicals, being involved in multitrophic interactions, can also be used to influence the behaviour of natural enemies of pests. Some or all of these activities can be utilised as components of integrated pest strategies (Agelopoulos *et al.*, 1999).

2.1.2.1.1 STRATEGIES FOR USE OF SEMIOCHEMICALS IN PEST CONTROL

The semiochemicals that have been used most successfully in pest control are lepidopterous sex pheromones and the aggregation pheromones of Coleoptera (Ridgway *et al.*, 1990). Many commercially developed systems exist for use of lepidopterous sex pheromones, either in monitoring systems or in slow release formulations to disrupt normal mate location. For control of forest pests, aggregation pheromones of bark beetles are used in trap-out procedures. However, semiochemicals, when employed alone, may give ineffective or insufficiently robust pest control and, alternative approaches must be considered (Agelopoulos *et al.*, 1999).

Semiochemicals will, in the future, find use within push-pull or stimulo-deterrent diversionary strategies (SDDS) (Pickett *et al.*, 1991). In such approaches, the harvestable crop is protected by means of semiochemicals such as plant-derived antifeedants, by employing repellent crop cultivars and by exploiting semiochemicals from non-host plants that interfere with location of the host plant by the pest. Aggregation of pests away from the crop is encouraged by attractants

such as sex and oviposition pheromones and by trap crops producing large quantities of host attractants. The trap crops can also be treated with a population-reducing component such as a highly selective pesticide, or a pathogenic biological control agent for which conditions on the trap crop can be modified to benefit its development. These semiochemically based control strategies should be designed to exploit natural populations of beneficial insects such as predators and parasitoids. Each component of the SDDS, when compared with conventional broad-spectrum toxicants, is relatively ineffective; this has the advantage of not selecting strongly for resistance and thus contributes to the overall sustainability of the approach (Agelopoulos *et al.*, 1999).

2.1.2.2 GENETIC RESISTANCE

Genetically there are three main types of resistance. **Monogenic resistance** is controlled by a single gene, usually a major gene that has a relatively large effect. This type of resistance is fairly easily incorporated into a breeding programme, and it usually gives a high level of resistance; but this resistance is just as easily 'broken' by new pest strains. **Oligogenic resistance** is the term used when the character is controlled by several genes acting in concert. **Polygenic resistance** is the result of many genes, and is clearly more difficult to incorporate into a plant-breeding programme. It may be either morphological or biochemical, and it is generally less susceptible to biotype resistance ('breaking'). Many of the genes will be minor genes which individually only have a small effect genetically (Hill, 1983).

One must keep in mind that resistance is the combined effect of all the genes of an individual; genes concerned primarily with resistance may also express themselves in diverse ways. Most of the resistance cases investigated would fall into the categories of oligo- and polygenic resistance. The division of resistance into these groups is popular among plant pathologists. Oligo- and polygenic resistance appears to be preferable to monogenic resistance as a strategy to safeguard against genetic vulnerability resulting from a breakdown of resistance caused by the

selection of new aggressive biotypes. The term **major gene resistance** is used synonymously with polygenic resistance. Since all oligogenes are not minor genes in the sense of being unimportant, both terms must be properly defined to avoid misleading connotations. Plants may vary continuously in resistance without necessarily falling into clearly defined groups. Single-gene effects are usually studied by measuring the damage to segregating plant populations challenged by unknown insect biotypes, or by evaluating the effect of the plant on the survival, growth, and reproduction of the insect.

Multiline resistance is the resistance conveyed by mixing phenotypically similar but genotypically dissimilar pure lines. The genotypic differences between component lines usually involve vertical resistance. A multiline is grown by mixing seeds of several resistant lines, which differ only in the resistance genes they carry. From the agronomic point of view, a field planted to a multiline need not appear different from a field planted to a genetically uniform cultivar, but to an insect population a multiline is a composite of different host genotypes. Resistance genes can be introduced into a multiline by adding component lines, derived from backcrossing resistant parents to an adapted standard cultivar as the recurrent parent (Horber, 1980).

In epidemiological terms, resistance is classified as either **horizontal resistance** (durable resistance), with a long-lasting effect and effective against all genetic variants of a particular pest, or **vertical resistance** (transient resistance), effective for a short period against certain variants only. There are a few other terms, which are in use in plant breeding for pest resistance. **Field resistance** is the term used commonly to describe resistance that gives effective control of a pest under natural conditions in the field. But it is difficult to characterise in laboratory tests; usually it is a complex kind of resistance giving only partial control. **Passive resistance** is when the resistance mechanism is already present before the pest attack, for example an especially thick cuticle, or hairy (pubescent) foliage. **Active resistance** is a resistance reaction of the host plant in response to attack by a parasite, more usually applicable to attack by pathogens rather than pests

(insects, etc.); for example, the formation of phytoalexins or other antibiotics by some host plants in response to attack by some pathogenic fungi. **Qualitative resistance** applies when frequency distribution of resistant and susceptible plants in the crop population is discontinuous, and the plants are easily categorised individually as either resistant or susceptible. **Quantitative resistance** is the term used when a crop shows a continuous gradation between resistant or susceptible plants within the population, with no clear-cut distinction between the two types (Hill, 1983).

The factors that determine the resistance of host plants to insect establishment include the presence of structural barriers, allelochemicals, and nutritional imbalance. These resistance qualities are heritable, and operate in a concerted manner and tend to render the plant unsuitable for insect utilisation (Panda & Khush, 1995). Mathes & Charpentier (1969) have postulated four main types of resistance to moth borers:

(1) Unattractiveness of a host plant to moths for oviposition, food or shelter (non-preference) (Mathes & Charpentier, 1969). Because the term 'non-preference' describes the response of the insect rather than a plant characteristic, it has been replaced by antixenosis, defined as plant properties evoking negative (non-preference) responses or total avoidance by insects (Schoonhoven *et al.*, 1998).

(2) Host-plant characters unfavourable for entry of borers into the plant (Mathes & Charpentier, 1969).

(3) Adverse effect of host-plant on borer development usually caused by certain nutritional and physiological characteristics of the plant tissue (Mathes & Charpentier, 1969).

Antibiosis, according to the terminology of Painter (1951), includes both (2) and (3). Antibiosis is the resistance mechanism that operates after the insects have colonised and have started utilising the plant (Panda & Khush, 1995). In this case the plant resists insect attack, and has an adverse effect on the bionomics of the pest by causing the death of the insects or decreasing their rate of development or reproduction (Hill, 1983). The antibiotic effects may result in a

decline in insect size or weight, reduced metabolic processes, increased restlessness, and greater larval or pre-adult mortality. Indirectly, antibiosis may result in an increased exposure of the insect to its natural enemies. Plants that exhibit antibiosis reduce the rate of population increase by reducing the reproduction rate and survival of insects. In certain cases, antibiosis cannot be clearly separated from antixenosis because of the extreme deterrent chemicals and/or physical factor(s) in the plant cultivar. Similarly, some morphological characteristics of the plant such as leaf trichomes or tissue toughness, are so critical for the insect to be able to react to their host plant, it is difficult to distinguish between antixenotic mechanisms of resistance. There are often overlaps between the morphological and biochemical bases of resistance. The antibiotic properties of the host plant may be expressed as constitutive or induced resistance against herbivores (Levin, 1976).

(4) Host-plant tolerance is the ability to repair, recover or withstand damage (Mathes & Charpentier, 1969). Tolerance is a genetic trait of a plant that protects it against an insect population that would damage a susceptible host variety, so that there is no economic yield loss or lowering of the quality of the plant's marketable product. This is characteristic of healthy vigorous plants, growing under optimum conditions, that heal quickly and show compensatory growth (Hill, 1983). Tolerance is often confused with low level of resistance or moderate resistance. Some varieties of crop plants may show both tolerance to a pest as well as antibiosis; this is true for several stalk borers (Hill, 1983). The mechanism of tolerance is distinct from antixenosis and antibiosis (Hill, 1983). Tolerance does not affect the rate of population increase of the target pest, but does raise the threshold level. Tolerance is an adaptive mechanism for survival of the plant, and is more or less independent of the effect upon the insect.

Pest avoidance is when the plant escapes infestation from the pest by not being at a susceptible stage when the pest species is at its peak. Some varieties of apples escape infestation by several different pest species in the spring by having buds that do not open until after the main emergence period of the pests, thus reducing the final amount of damage inflicted. These

functional categories of resistance do not exclude each other, but may interact, complement, and compensate for each other along with other biotic communities and abiotic factors in reinforcing the expression of resistance.

2.1.2.2.1 CHEMICALLY BASED RESISTANCE

Present knowledge clearly indicates that chemically based resistance is a major component of the plant's total defence armament against herbivores. It is diverse in composition and extremely effective ecologically, at least if humankind does not interfere. It seems clear that we must achieve new levels of awareness, and practice of the derived knowledge, if genetic engineering is to be used effectively to alter both the pathways and timetables of evolution of chemically based plant defences for our benefit (Kogan & Paxton, 1983).

2.1.2.2.2 GENETIC SOURCES

Success in identifying sources of resistance is directly related to the diversity of germplasm available and the probability of resistance occurring in the host populations. The search for sources of resistance is carried out in a logical sequence: first in adapted cultivars, then in plant introductions and exotic germplasm, and finally in near relatives of the cultivar. The identification of the sources of resistance is followed by hybridization, selection in segregating generations, and progeny testing. Resistance is frequently found in primitive cultivars or related species. The transfer of resistance from these exotic sources may require the use of special manipulations such as cell culture. An excellent example of the transfer of resistance from one species to another is the incorporation of greenbug resistance in wheat from rye. The advances made in basic science, through developments such as cell and embryo culture, have a marked impact on progress in the applied science of breeding resistance to insects (Ortman & Peters, 1980).

The International Board for Plant Genetic Resources (1976) published a priority list for crops and regions. Their criteria for priority areas were as follows: (1) the risk that genetically diverse materials will be lost owing to changes in land use; (2) the economic and social importance of the materials to be collected; (3) the recognised requirements of plant breeders for genetically diverse materials; and (4) the size, scope, and quality of existing collections. Plant exploitation and collection is a critical activity as scientists continue to seek and utilise naturally occurring sources of resistance.

Allard (1970) observed that each species contains millions or even hundreds of millions of variants, so sampling is a challenge. Unfortunately, Harlan (1972) was generally correct when he observed, in no collection is there an adequate sampling of the spontaneous races that are most likely sources of disease and insect resistance.

One frequent shortcoming of plant exploitation activities is the lack of an entomologist as a member of the team. Thus the potential for success is a function of the variation in both insect and host, coupled with the frequency of occurrence of the plant variants in the population and subsequent identification and utilisation of variants (Ortman & Peters, 1980).

2.1.2.3 ECOLOGICAL RESISTANCE

Ecological resistance has been categorised as pseudo-resistance because it results, not from the genetic characters inherent in the host plant, but from some temporary shifts in the environmental conditions favourable to the otherwise susceptible host plants (Painter, 1951). Although some factors contributing to pseudo-resistance are fortuitous and unusual, plant varieties that exhibit pseudo-resistance are of considerable importance in pest management systems and deserve special consideration. Another category of ecological resistance, induced resistance, occurs in response to damage by pathogens, herbivores, environmental stress, or specific chemical and physical treatment (Rhoades, 1979).

2.1.2.3.1 PSEUDO-RESISTANCE

Alterations in plant growth patterns that result in asynchronies of insect-plant phenologies constitute a modality of resistance known as pseudo-resistance. Certain crop varieties may overcome the most susceptible stage rapidly and thus avoid insect damage. Early-maturing crop cultivars have been used in agriculture as an effective pest management strategy. However, plants that evade insect attack by this mechanism are likely to be damaged if the pest populations build up early (Painter, 1951).

2.1.2.3.2 INDUCED RESISTANCE

Induced resistance is the qualitative or quantitative enhancement of the plant's defence against invading organisms in response to pest-related injury or extrinsic physical or chemical stimuli. The extrinsic stimuli are known as inducers or elicitors. The injury-dependent responses of plants are components of induced resistance (Kogan & Paxton, 1980). Induced resistance can also result from an environmental change that may lead to a temporary benefit for the host plant. The application of fertilizers, herbicides, insecticides, growth regulators, and mineral nutrients, or a variation in temperature and day length, or insect and pathogen attack can all change the chemical constituents of plant tissue, and consequently their nutritional value for pests (Karban, 1991).

2.1.2.4 MORPHOLOGICAL BASES OF RESISTANCE

Morphological (physical) resistance factors interfere physically with locomotor mechanisms, and more specifically with the mechanisms of host selection, feeding, ingestion, digestion, mating, and oviposition as opposed to those factors affecting chemically mediated behavioural and metabolic processes discussed above. However, due to certain characteristics, the plant may not be utilisable and may deter the insects. Insects are noticeably reluctant to colonise some individual plants, or some particular strain of host-plant, and these plants seem to be less

attractive to the pest by virtue of their texture, colour, odour or taste (Hill, 1983), trichomes, surface waxes, silication, or sclerotization of tissues (Kogan & Paxton, 1980).

In certain situations, although the insects may come in contact with the plant, the antixenotic characteristics of the plant do not allow the insect to colonise. Plants that exhibit antixenotic resistance should have a reduced initial number of colonisers early in the season; the size of the insect population should also be reduced after each generation as compared with susceptible plants. Sometimes, the antixenosis mechanism is so effective that the insects starve and die (Painter, 1968). The deterrent mechanisms influence an insect's behavioural response to the plant. In addition, allomones affecting insect behavioural and metabolic processes may occur in plant morphological structures (trichomes or bracts). Thus, chemical and morphological resistance factors intertwine in a continuum of defence (Kogan & Paxton, 1980).

Host plant characteristics including morphological, physical, or structural qualities interfere with insect behaviour such as mating, oviposition, feeding, and food digestion. While selecting their hosts, insects respond to various plant-stimuli and the presence of repellents, antifeedants, or feeding deterrents contribute to antixenotic types of resistance. Critical observation on a number of phytophagous insect species showed that before feeding on a plant they make some sensory exploration of the plant surface as a prelude to biting. As a mechanism of resistance, antixenosis may represent one or more breaks in the chain of responses leading to oviposition or feeding. These breaks take place in three forms: (1) the absence of an arrestant or attractant, (2) the presence of a repellent, or (3) an unfavourable balance between an attractant on the one hand and a repellent on the other. The relevant chemistry of the host plant seems to influence the herbivore's acceptance or rejection for oviposition or food (Schultz, 1988).

The modalities of plant resistance against different feeding guilds of insects seem to differ. The plant surface is embedded with physical and chemical factors responsible for antixenosis to feeding insects (Southwood, 1986). Plants may alter the levels and balance of compounds that

serve as insect feeding stimulants and deterrents, which results in the intimate associations becoming behaviourally/physiologically unacceptable. Each plant species has a unique set or collection of defence traits ranging from morphological to phytochemical parameters that have behavioural and physiologic ramifications for a potential herbivore consumer. The phytophagous insects must be able to locate the most suitable nutritional substrates among the multitude of plant species available within its temporal and spatial environment. These behavioural patterns of insects can be adversely affected by antixenotic mechanisms involving physical and biochemical factors of the respective host plants (Panda & Khush, 1995).

2.1.2.5 INSECT BEHAVIOUR AND PLANT RESISTANCE

2.1.2.5.1 HAZARDS OF NEONATE SURVIVAL

The fecundity of any biological population tends to outstrip the long-term capacity of the habitat to support an expanding population. As a result, most neonate individuals face fearsome odds against their survival to the reproductive stage. Among insects, where the biotic potential is generally very high, the attrition rate normally exceeds 95%. Exceptions occur when a species is introduced into an environment that is partially devoid of the biotic and abiotic factors that normally hold the population at a stable equilibrium. These result in damaging outbreaks of the species, be it insect, vertebrate, plant, or micro-organism. Agriculture tends to encourage the increase of insect populations to damaging levels by virtue of ecosystem disruption and the maintenance of artificially large host plant monocultures, frequently of genotypically uniform composition (Beck & Schoonhoven, 1980).

The neonate phytophagous insect is confronted by an array of factors unfavourable to its survival. Many of these factors lie outside the purview of the present discussion; these include the non-biological density-independent influences of temperature, rainfall, soil type, and so on, as well

as density dependent biological factors such as disease, predation, and intraspecific competition (Beck & Schoonhoven, 1980).

Even the most susceptible host plant of a given insect is not defenceless, and only a small percentage of the feeding stages of the insect will survive. Many studies of the resistance of maize, *Zea mays*, to the European corn borer, *Ostrinia nubilalis*, have employed the inbred cultivar 'WF9' as the "standard susceptible" genetic line, and have compared other genetic lines to it for resistance. Under protective laboratory conditions, more than 80% of the newly hatched borer larvae succumbed within 6 days when reared on seedlings of 'WF9' (Beck & Lilly, 1949). They also found that an age-related increase in susceptibility to the borer occurred only slightly more rapidly in 'WF9' than in more resistant inbred lines tested. From a dietetic standpoint, host plants are generally inferior to well-balanced, nutritionally complete laboratory culturing media. Several species of phytophagous Lepidoptera have been found to grow faster, to a larger body mass, and with better fecundity and longevity on artificial dietary media than on host plant tissues (Beck, 1974). Such media are devoid of physical and chemical plant defence factors, but may also lack some sensory factors, such as attractants and stimulants, that may be important to survival under natural conditions (Beck & Schoonhoven, 1980).

2.1.2.6 PREVIOUS WORK ON LARVAL ANTIXENOSIS/ANTIBIOSIS TO SUGARCANE BORERS

The leaf surface acts as the crucial interface between the insect's battery of chemoreceptors and the plant (Southwood, 1986). Hence, antixenosis for feeding in plants is composed of the glandular and non-glandular trichomes, leaf-surface chemicals, tissue toughness, nutrient deficiency and constitutive chemicals (repellents and deterrents) (Panda & Khush, 1995).

Meagher *et al.* (1996) compared laboratory measures of larval and adult antixenosis and antibiosis of sugarcane with results of field injury so that mechanisms involved in conferring plant

resistance to the Mexican Rice Borer, *Eoreuma loftini* can be determined. They found that larvae function well once they have entered leaf sheaths but have difficulty becoming established in leaf sheaths, entering stalks, or tunnelling within stalks. Low larval numbers found on pieces of leaf sheath of the variety CP 70-321 provided evidence for low larval establishment in leaf sheaths. The opposite situation was true for LCP 81-10, a genotype possessing high stalk injury. Diet mixtures with this genotype produced small larvae and pupae and long development times, but larval establishment, as indicated by numbers of larvae on leaf sheath pieces, was comparable with NCo310. Perhaps stalk admittance and consumption by *E. loftini* on LCP 81-10 is more efficient than on other genotypes. CP 70-324, a genotype possessing field resistance, showed evidence for leaf sheath antibiosis and a trend for ovipositional antixenosis, but provided no evidence for larval antixenosis (Meagher *et al.*, 1996).

The lack of foliar establishment and mortality of neonate larvae has been described as a major factor of resistance (Kyle & Hensley, 1970; David & Joseph, 1984), with leaf sheath appression, the ability of a plant to self-trash (shed lower leaves and leaf sheaths), and leaf midrib hardness documented as specific resistant characters. However, larval foliar establishment as a resistance factor among cultivars becomes important only if these differences persist until stalks are invaded (Bernays *et al.*, 1983); if over longer periods of development, the final level of infestation is independent of initial numbers, then differences among cultivars in establishment are not important (Chapman *et al.*, 1983). Larval resistance can be separated into "leaf" and "stalk" resistance. Neonate and young larvae must be able to become established within the leaves, midribs and leaf sheaths and obtain sufficient nutrients before entering stalks (Meagher *et al.*, 1996).

The lack of antibiosis determined in laboratory tests by Meagher *et al.* (1996) on sugarcane against *E. loftini* indicates that larvae function well once they have entered leaf sheaths, but have difficulty becoming established in leaf sheaths, entering stalks, or tunnelling within stalks. Low

larval numbers found on pieces of CP 70-321 (resistant cultivar) leaf sheath provided evidence for low larval establishment in leaf sheaths. The opposite was true for LCP 81-10 (susceptible cultivar). Diet mixtures with this genotype produced small larvae and pupae and long development times, but larval establishment, as indicated by numbers of larvae on leaf sheath pieces, was comparable with NCo310. Perhaps stalk admittance and consumption by *E. loftini* on LCP 81-10 is more efficient than on other genotypes. CP 70-324, a genotype possessing field resistance, showed evidence for leaf sheath antibiosis and a trend for ovipositional non-preference, but provided no evidence for larval non-preference. Results obtained by these researchers confirmed that several mechanisms of stalk borer resistance, including antibiosis and non-preference, are present across sugarcane genotypes (Meagher *et al.*, 1996).

David & Joseph (1984) conducted studies on the mechanism of resistance in the original clones and commercial varieties against the internode borer, *Chilo sacchariphagus indicus* (internode borer). These studies were conducted in filed and cage conditions with selected resistant and susceptible host plant materials. Restlessness of internode borer larvae was a marked feature in the early stages. This was indicated by their unsettled behaviour during early feeding on the tissues and remaining inside the leaf spindle or inside the leaf sheaths. The lowest percentage of larvae remaining restless was in *Saccharum officinarum*. The highest percentage of restlessness was found in *Saccharum spontaneum* which was at par with *Saccharum robustum*. The same trend was observed in the commercial varieties where in susceptible varieties Co. 419 and Co. 6304 had less percentage of restless larvae while the resistant varieties Co. 975 and Co. J. 64 had significantly higher percentage of the same. Migration of internode borer larvae during the growth period from the damaged internode to another internode in the same cane or adjoining cane is a common phenomenon. The larval survival assessed at 15^{th} and 30^{th} day after release gave indication as to the number of larvae in the respective hosts that could survive and pupate (David & Joseph, 1984).

It was observed that the mean number of internode borer larvae, both at 15th and 30th day, after inoculation was maximum in *S. officinarum* and differed significantly from the mean larval population for both the periods in *S. barberi*. The mean number of internode borer larvae that survived was significantly low in *S. spontaneum* and *S. robustum*. The mean number of surviving larvae was the maximum at the 15th day after inoculation in Co. 6304 followed by Co. 419. The difference between them was significant. However, on the 30th day after inoculation even though the same trend was evident, the difference between the values for these varieties was not significant.

Neonate larvae display restlessness before settling down for feeding and this is more evident in resistant *S. spontaneum*, Co. J. 46 and Co. 975. This may be due to the hardness of the rind or tight leaf sheath. Significantly higher percentage of larvae survived in susceptible *S. officinarum*, Co. 419 and Co. 6304 than in resistant clones and varieties. This difference narrows down at 30th day. This shows that the antibiotic effect is more pronounced in the early larval period than in the late larval period. Prolongation of larval period in the resistant clones and varieties may be due to inadequate nutrition (David & Joseph, 1984).

Sosa (1988) studied the effects of pubescence on oviposition and mobility of *Diatraea saccharalis* using leaves from 25 plants from each of two clones of *Saccharum robustum* sugarcane plants, the pubescent 'NG 77-147' and the glabrous 'NG 77-195.' In the free-choice and no-choice tests with excised leaves, significant differences between the pubescent and glabrous clones occur only in the mean number of egg masses laid on leaves in the free-choice test. This was higher on the pubescent clone. In the free-choice test, he observed significantly more eggs and egg masses laid on the abaxial leaf surface of the pubescent clone. However, significantly more eggs were laid on the adaxial leaf surface of the glabrous clone than on any other leaf surface.

Pubescence may have caused moths to move more often in a search for a suitable surface for oviposition, which could account for pubescent leaves receiving a higher number of egg masses

but fewer eggs per egg mass. In no-choice test, the adaxial leaf surface of the glabrous clone received significantly more eggs than any other surface; the numbers of egg masses and eggs per egg mass were also significantly higher on the adaxial leaf surface. Overall, oviposition by the sugarcane borer was higher on the glabrous clone than on the pubescent clone. Leaves of the glabrous lone received the most eggs, egg masses, and eggs per mass; often, these differences were significant and first-instar sugarcane borers travelled a mean of 17.5 mm in 30 s, which was significantly more than the distance of 9.9 mm travelled on the pubescent clone. Thus, level of pubescence adversely affected oviposition and mobility of sugarcane borers (Sosa, 1988).

In another study, Sosa (1990) compared oviposition by *D. saccharalis* on various substrates. When leaf surfaces of sugarcane cultivars (CP 70-1133 and CP 72-355—*Saccharum* spp.; NG 77-195 and NG 77-147—*S. robustum*), maize cultivar Golden Corn Bantam T-51, sorghum cultivar Rio, and rice cultivar Lemont were compared, the borer laid significantly more eggs on the sugarcane clones than on the other crops. More eggs were laid on the leaf surfaces of glabrous sugarcane clones than on a pubescent clone. These data support earlier work (Sosa, 1988) that showed that pubescence adversely affected oviposition by *D. saccharalis*. Pubescence appears to be an important morphological character conferring resistance in sugarcane to this borer (Sosa, 1990).

Predictive models based on data acquired by near infrared (NIR) spectrophotometry suggest that components in extracts from sugarcane nodal budscales contribute towards resistance to *E. saccharina* (Rutherford, 1998). NIR spectra for 60 sugarcane clones varying in resistance to *E. saccharina* indicated that chlorogenates and flavonoids might be involved in the NIR calibration and also in the interaction between this insect and sugarcane. Two extreme types of flavonoid profiles subsequently were revealed, one associated with resistance and the other with susceptibility (Rutherford, 1998). Incorporation of the susceptible type profile into a defined synthetic diet increased feeding initiation and subsequent survival of first instar larvae compared

with the resistant type profile (Rutherford, 1998). NIR calibrations proved capable of predicting the resistance ratings of validation sets. Based on the implicated involvement of aromatics by NIR spectral data and correlated flavonoids profile differences, chlorogenates and flavonoids would appear to be involved with resistance and/or susceptibility to *E. saccharina* in sugarcane (Rutherford, 1998).

In southern Africa plants of the families Gramineae, Cyperaceae and Juncaceae have been found to be indigenous host plants of *E. saccharina* (Conlong, 2000). These families are shown to be closely related phytochemically, based on flavonoid components (Harborne *et al.*, 1985). Flavonoids are involved in many plant-insect interactions and can be active in host-plant recognition, feeding stimulation or deterrency, as well as having effects on insect physiology and nutrition (Slansky, 1992).

Nuss & Atkinson (1983) assessed attractiveness of sugarcane plants to an ovipositing moth and larval performance, measured by the number and biomass of larvae. They found most eggs on variety N11 in the first experiment, but in the subsequent experiments there were fewer eggs on N11 than on NCo376 or N12. Experiment 1 had the most eggs per pot despite the release of fewer moths than in subsequent experiments. This experiment was conducted in March when temperatures were higher than those in Experiments 2 and 3, which were conducted in July/August, and that in Experiment 4 which was done in October. The cooler weather may have reduced moth activity. There was large variation in larval numbers, mass, biomass and width of the head capsule of *E. saccharina* larvae. Variability was large in both moth preference and larval performance trials. The method employed to assess larval performance appears to be a useful means of determining varietal susceptibility because significant differences between varieties were obtained (Nuss & Atkinson, 1983).

Larvae were placed on dead leaf material in the centre of circles of differing diameters and assessments then made, at different times after hatching. The highest recoveries (nearly 60%) were

from those circles having the smallest diameter (200 mm), while the lowest (maximum of 12%) were from the circles of greatest diameter (1600 mm). A few individuals were able to travel considerable distances, but most seemed to restrict their dispersal to within 200 mm of hatching point. However, larvae in litter cover a much greater distance than the radius of the circles used. Most females lay their eggs close to the stalks making it easy for most larvae to reach them. These results suggest that the dispersal range of most larvae in cane is small, although adequate to locate host plants (Leslie, 1993).

In a three-year study conducted by White (1993), numbers of larvae and pupae recovered after 30 days were low and variable among cultivars CP 74-383 (susceptible), CP 65-357 (intermediate) and CP 70-321 (resistant). In 1987, only 38 of the total of 450 (8.4%) larvae released were recovered at 30 applied per sampling date from CP 74-383, 22 (4.9%) from CP 65-357, and 20 (4.4%) from CP 70-321. In 1988 and 1989, the total number of larvae and pupae recovered from the total of 540 ranged from a low of 19 (3.5%) for CP 70-321 to a high of 55 (10.2%) for CP 74-383 (White, 1993). His results suggested that sugarcane borer larvae are less selective at the leaf-feeding stage (instars 1-3), but require younger internodes for acceptable entry sites as they begin to establish themselves within the stalk (instars 4 & 5). Ring *et al.* (1991) found similar behaviour by the Mexican rice borer, *E. loftini* (Dyar). Larvae of this pest migrated from oviposition sites at the base of the stalk to green leaf sheaths at the top, ultimately to penetrate and complete development within the stalk. Larvae were found to prefer 10-day-old internodes as penetration sites. The pattern of establishment of larvae on the susceptible cultivar, CP 74-383 and intermediate cultivar, CP 65-357 was somewhat more consistent from year to year, compared with establishment on the resistant cultivar CP 70-321. The majority of the larvae established on the K+ 0 and K + 2 internodes on CP 74-383 and CP 65-357, while larvae became established in internodes K – 1 through K + 3 on CP 70-321. The youngest leaf with exposed dewlap, the node supporting this leaf, and the internode below this node were all designated K + 1. The next older

leaf, its supporting node and the internode below that node were designated K + 2, and so forth (White, 1993).

CHAPTER 3: GENERAL MATERIALS AND METHODS

3.1 PLANTING OF SUGARCANE VARIETIES FOR GLASSHOUSE EXPERIMENTS

Six sugarcane varieties: N11, N26 [N16] (susceptible); N17, NCo376 (intermediate) and N12, N21 (resistant) were planted, in preparation for budscale manipulation (possible role of the budscale in affecting stalk penetration and survival of larvae), rind hardness, epicuticular waxes and mobility experiments. Seed cane was obtained by cutting stalks into small sections (single-budded setts) that included the node, using a mechanical cutter. Setts were soaked in a solution of 5-ml Eria® (a fungicide) and 5 litres of water for 5 minutes to prevent fungal attack. Seed cane was planted in trays with 72 cells (buds facing up). The trays were placed in a germination room until shoots were visible. Thereafter trays were taken to the nursery until the cane was strong enough to be transplanted. A fertilizer (4:1:1 (45) N:P:K) was mixed with the sand before planting and one seedling was transplanted into each pot. Basins with holes fitted with stoppers (to enable easy removal of algae and dirt) were placed under the pots to retain irrigation water and avoid loss of nutrients. A timer was set for drip irrigation over 15 minutes, three times a day.

Thirty-six plants (six of each variety) were planted in pots and placed in the glasshouse to be used to test for the success of stalk penetration and larval growth associated with point of entry on the node. Pots were placed in nine rows with 12 pots in each row (18 pots/variety) using three Latin squares for three of the four experiments mentioned above. The plants for the mobility experiments were planted in the shadehouse because they had enough time to grow (only used in June 2000). Their pots were also arranged in a Latin square.

Pots were filled with river sand and seedlings were transplanted from trays into the pots (one plant per pot). These plants were placed in the glasshouse to speed growth due to the time in which they were planted (winter). They were then moved into the shadehouse on the first week of September to strengthen the stalks, by growing them under normal conditions. Plants were used when they were at least nine-months-old (average maturity age of cane), from February 2000.

3.2 LABORATORY EXPERIMENTS

3.2.1 STALK SEGMENTS IN JARS FOR SCREENING EXPERIMENTS

Six stalk segments were cut from each of the following varieties using a mechanical cutter: N11 and N26 [N16] (susceptible); NCo376 and N17 (intermediate); and N12 and N21 (resistant). One stalk segment represented a replicate, giving six replicates for each of the six varieties. Segments were obtained from the bottom section of each stalk, which is usually the mostly infested area (by *E. saccharina*) (Girling, 1972). The segments were not the same length (the average length was 20cm), because of the different lengths of the internodes caused by varietal and age differences amongst the plants. Two segments were cut from each stalk. Ends were sealed with thick melted wax to prevent larvae penetrating on cut ends and entry of fungi. Stalk sections were placed in 750ml plastic jars and supported with melted wax in an upright position, one stalk per jar. A hole was made in each lid and then sealed with neonate-proof gauze to prevent larvae from escaping and to allow air to pass through. Jars were arranged in a Latin square.

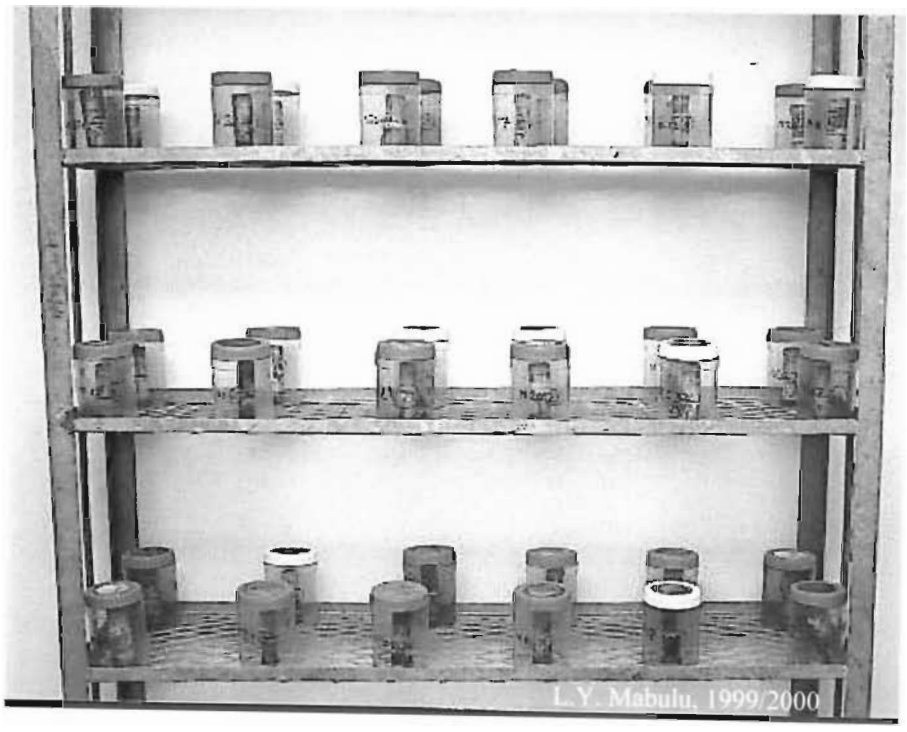

Figure 1: Set-up for screening experiments: each jar has a stalk segment with a node and five larvae released from the top.

Each stalk was inoculated with five larvae, by placing larvae on top of the segment with a small paintbrush. The temperature in the room was maintained at 26^0C and humidity at 75%. Larvae were left to feed for 14 days, after which the stalks were dissected and position of entry; number of surviving larvae; number of dead larvae and collective mass of larvae was measured for each segment.

This free-choice type of experiment was designed to allow larvae to choose the points of entry. This would clearly indicate which parts of the stalk neonate larvae prefer.

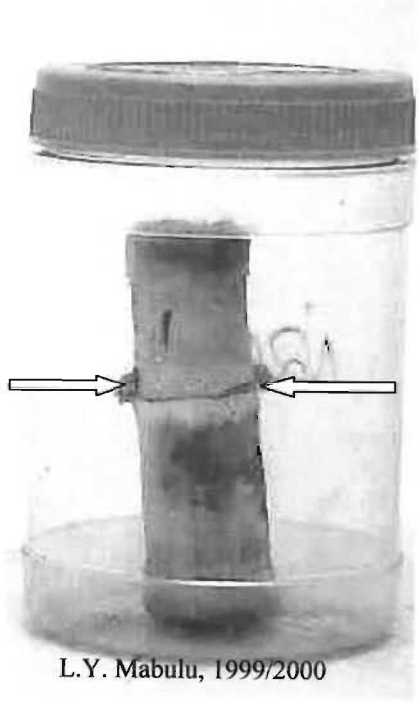

Figure 2: Close-up of jar used in screening experiments showing frass on either side of the stalk produced by larvae penetrating through the buds and leaf scars.

3.2.2 SUCCESS OF STALK PENETRATION AND LARVAL GROWTH ASSOCIATED WITH POINT OF ENTRY ON THE NODE

All plants were grown in the field and uninfested stalks chosen for the experiments. Assays were conducted in a metal cage (1m x 1m x 1m) covered with gauze to minimise loss of moisture from the segments.

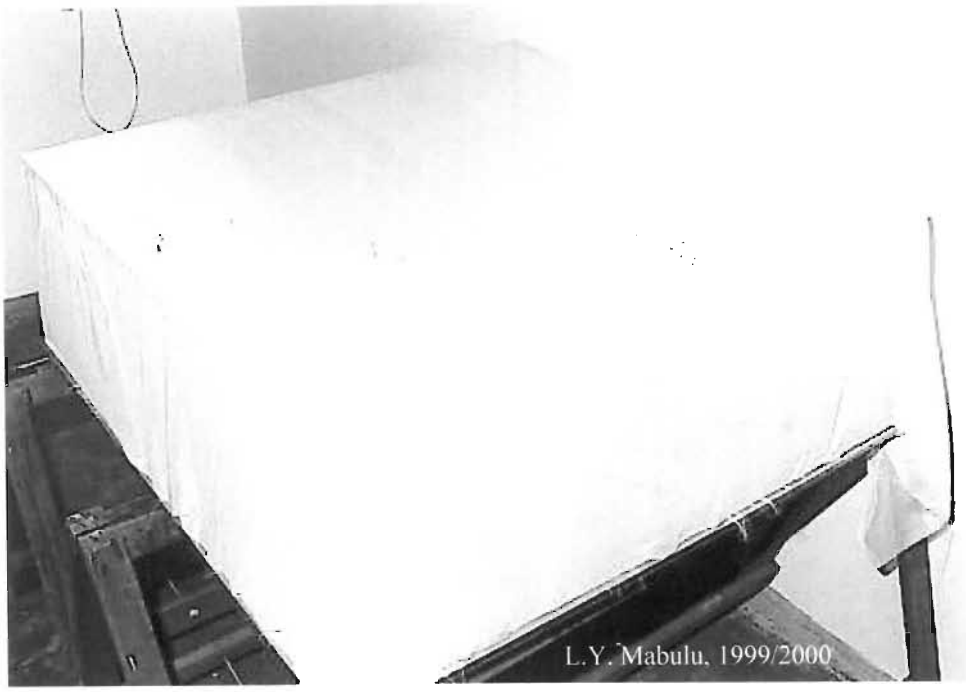

Figure 3: Metal cage used for budscale, rind hardness, root primordia and epicuticular wax experiments.

Stalk segments with two nodes were obtained from the standard six varieties. Thirty-six stalk segments were placed horizontally in the cage in a randomised complete block. Stalks containing bored internodes were not used, to control for any possible effects –mostly unknown– that prior infestation might have. Each stalk was considered a replicate. Each segment was sealed with wax at the ends to prevent entry of fungus and moisture loss. Seventy-two microcentrifuge tubes (1.5ml) with snap-on caps were used to confine larvae on two specific sites on each stalk segment, one of which was the control and the other the treatment. The tip of each tube was cut out

at the 0.5 ml mark, leaving a hole through which larvae could access the stalk. A hole was made with a hot needle on each cap and then sealed with neonate-proof gauze to provide air. At harvest, stalks were dissected and the number of live and dead larvae and their masses recorded. In these experiments larvae were not allowed any choice; they were confined to one part of the node to test if they would still feed even when they are not given a choice of different substrates. All data were subjected to One Way Analysis of Variance using SigmaStat 3.0.

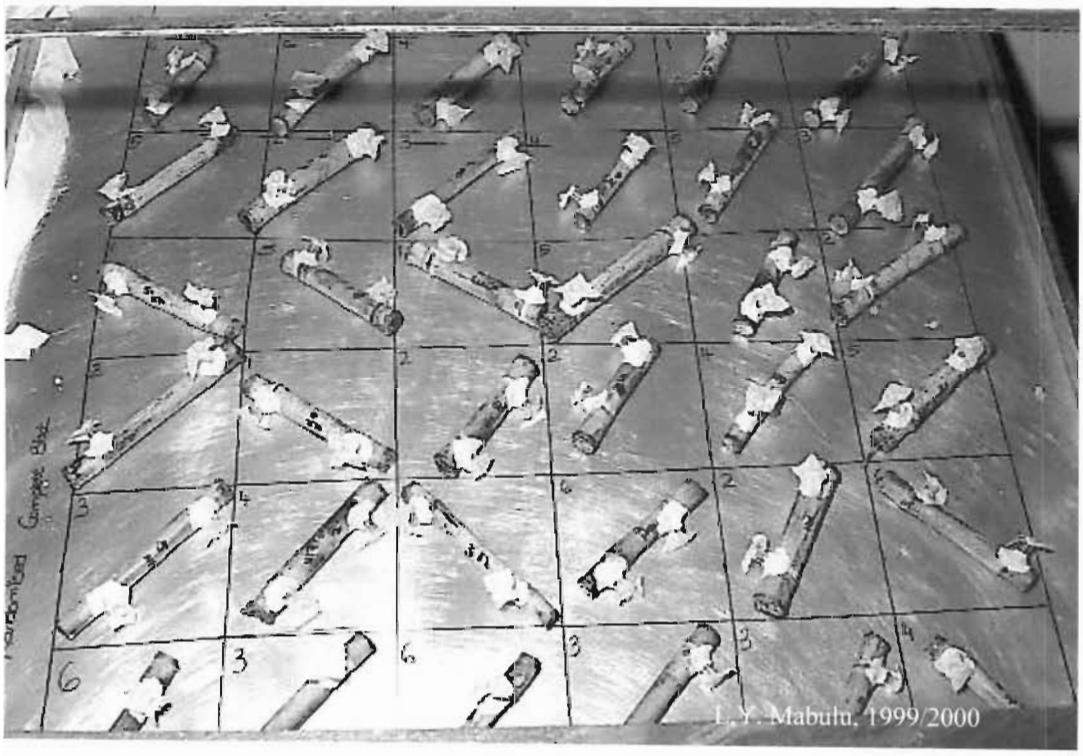

Figure 4: Cage set-up showing the randomised arrangement of stalk segments in all cage experiments.

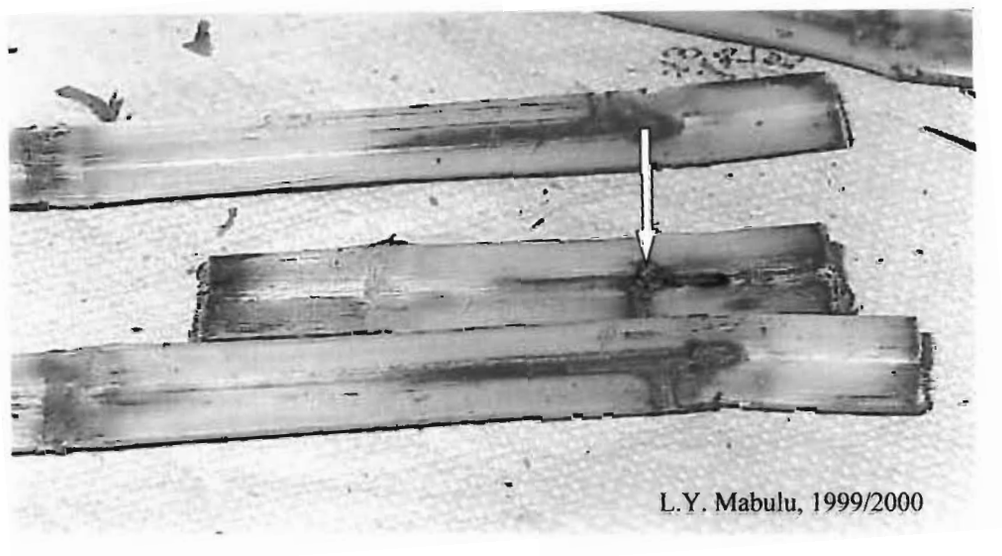

Figure 5: Dissected stalks from cage experiments, exposing the feeding larvae (shown by the arrow).

CHAPTER 4: SCREENING FOR RESISTANCE

In the past it has been determined that *E. saccharina* survival numbers are decided within the first week after egg hatching. And that during this time first-instar larvae are still on the stalk surface (Nuss, personal communication). Consequently, it is possible that plant surface chemistry might be involved in resistance to *E. saccharina* attack (Rutherford *et al.*, 1993). Woodhead and Padgham (1988) found that the surface wax of sorghum and rice contributed towards the resistance of these plants to the brown planthopper, *Nilaparvata lugens*.

4.1 USING STALK SEGMENTS WITH ONE NODE AND TWO NODES

4.1.1 METHODS

Stalk segments with one node and average lengths of 15 cm were used. Each stalk segment had one node. Larvae were observed immediately after release until they disappeared into the stalk segments and then left to feed for 14 days. After 14 days the stalks were dissected and larvae within them were weighed collectively for each stalk segment.

Stalk segments with two nodes were then used to allow larvae more penetration points and to assess the behaviour when the same number of larvae were given more entry points. The internodes on either side of the nodes were cut to almost the same length, with an average length of 25 cm.

4.1.2 RESULTS AND DISCUSSION

There were no significant differences in the masses of larvae among the six varieties using either one (survival; $H=3.896$, $P=0.565$ and larval mass; $F=0.606$, $P=0.666$) (Fig. 6 & 7, respectively) or two nodes (survival; $F=0.797$, $P=0.568$ and larval mass; $F=2.453$, $P=0.078$) (Fig. 8 & 9, respectively). Ease of stalk penetration in all varieties was not enhanced through the

presentation of two nodes as opposed to one. Thus larval development did not differ significantly for any of the varieties used although trends were apparent.

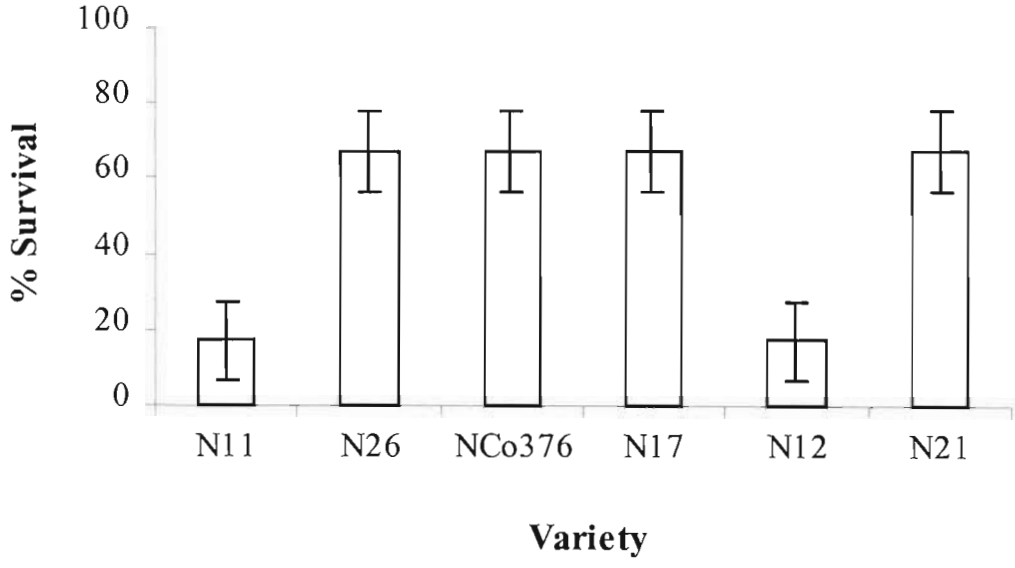

Figure 6: Percentage survival of larvae (bars = Standard Error) recovered from stalks with one node after 14 days.

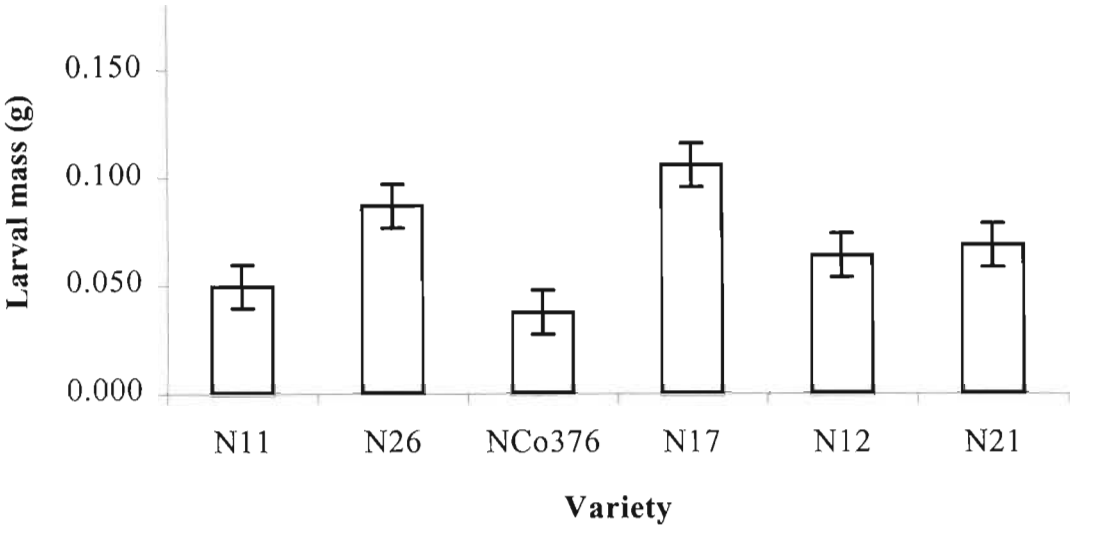

Figure 7: Mean 'combined mass' (bars = Standard Error) of larvae recovered from stalk segments with one node after 14 days.

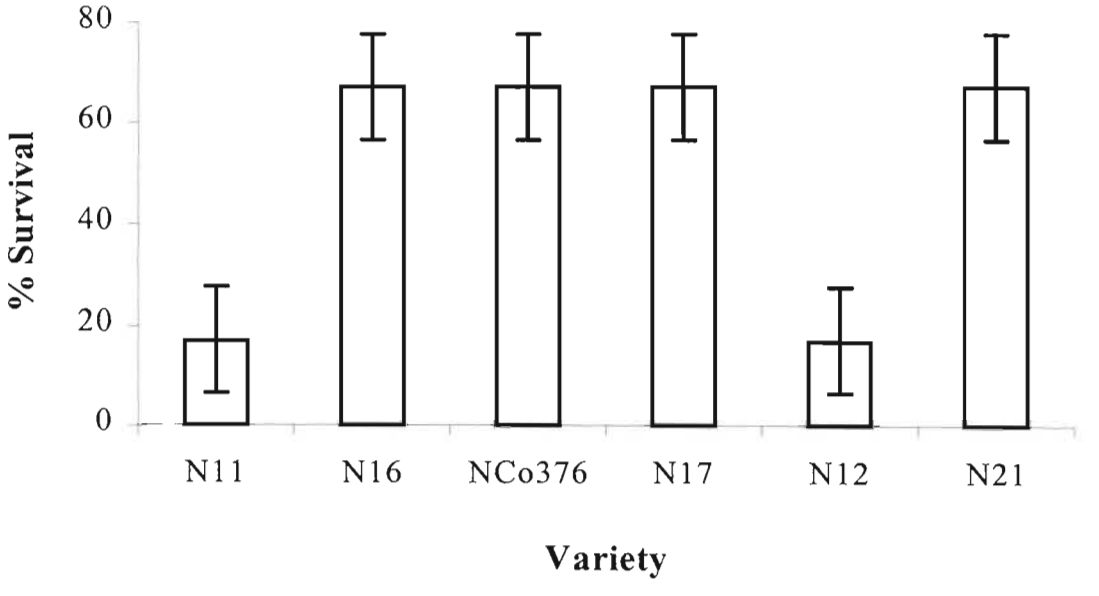

Figure 8: Percentage survival of larvae (bars = Standard Error) recovered from stalks with two nodes after 14 days.

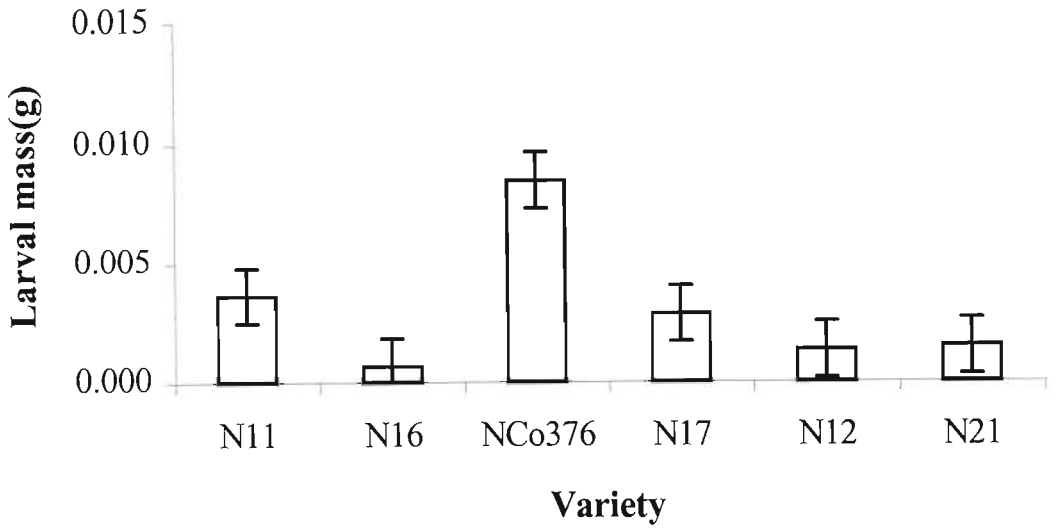

Figure 9: Mean mass (bars = Standard Error) of larvae recovered from stalk segments with two nodes after 14 days.

The results indicate that use of stalk segments in laboratory bioassays for resistance to *E. saccharina* would not produce reliable results as a mass screening method, especially when large numbers of unknown clones were involved.

Meagher *et al.* (1996) conducted tests to compare laboratory measures of larval antibiosis and adult and larval non-preference with results of field injury so that mechanisms involved in conferring plant resistance to the Mexican rice borer, *Eoreuma loftini* could be determined. Larvae showed preferences for foliar establishment in certain genotypes, and it appeared that larval preference might be locationally directed among different leaf sheaths within a stalk.

Larval foliar establishment among cultivars as a resistance factor becomes important only if these differences persist until stalks are invaded (Bernays *et al.*, 1983). With longer periods of development, the final level of infestation is independent of initial numbers, and then differences among cultivars in establishment are not important (Chapman *et al.*, 1983). The lack of antibiosis

determined in laboratory tests indicated that larvae functioned well once they had entered leaf sheaths, but have difficulty becoming established in leaf sheaths, entering stalks, or tunnelling within stalks. Low larval numbers found on pieces of CP 70-321 (resistant cultivar) leaf sheath provided evidence for low larval establishment in leaf sheaths. The opposite was true for LCP 81-10 (susceptible cultivar). Diet mixtures with LCP 81-10 produced smaller larvae and pupae and longer development times, but larval establishment, as indicated by numbers of larvae on leaf sheath pieces, was comparable with NCo310. Perhaps stalk admittance and consumption by *E. loftini* on LCP 81-10 is more efficient than on other genotypes. CP 70-324, a genotype possessing field resistance, showed evidence for leaf sheath antibiosis and a trend for ovipositional non-preference, but provided no evidence for larval non-preference. Results obtained by these researchers confirmed that several mechanisms of stalk borer resistance, including antibiosis and non-preference, are present across sugarcane genotypes.

4.2 NODES AND INTERNODES AS POINTS OF LARVAL STALK PENETRATION

4.2.1 METHODS

Two sets of experiments were conducted at the same time. In one, the stalk segments included the node as in 4.1 above. In the other, only the internodes were used without the nodes. Thus having the two experiments running at the same time and under the same conditions to make a comparison of the larval preference for nodes and internodes.

4.2.2 RESULTS AND DISCUSSION

The differences in larval masses among varieties were non-significant (F=1.437, P=0.264) (Fig. 11).

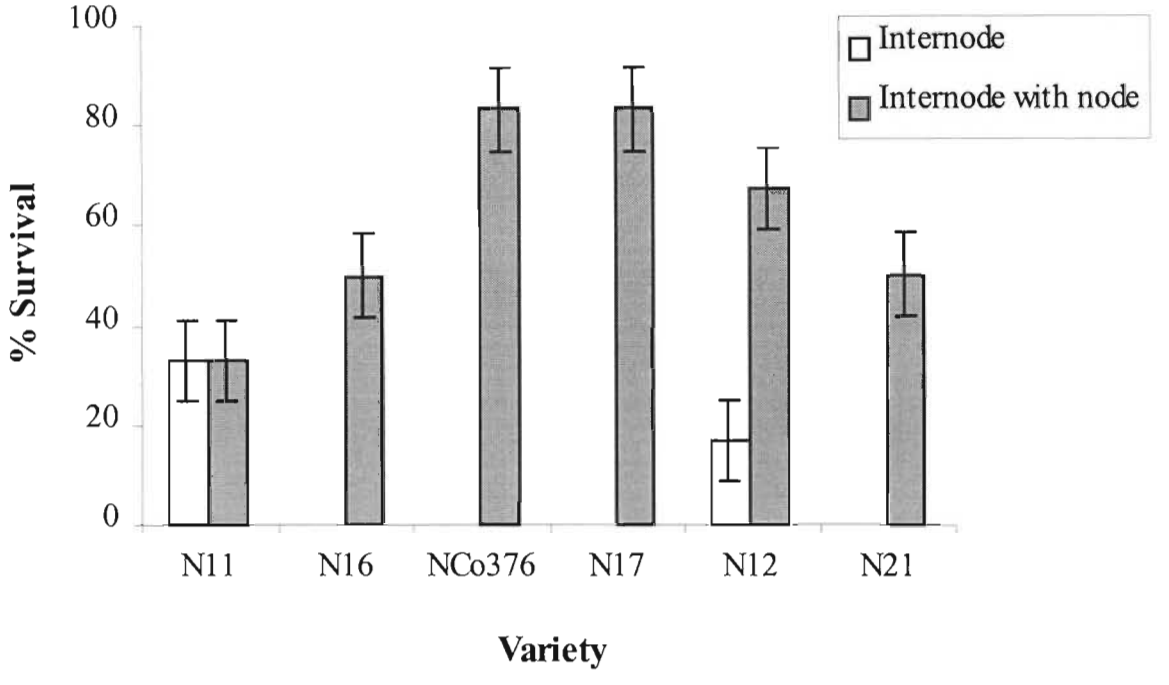

Figure 10: Percentage survival of larvae (bars = Standard Error) recovered from the internodes & the internodes with nodes after 14 days.

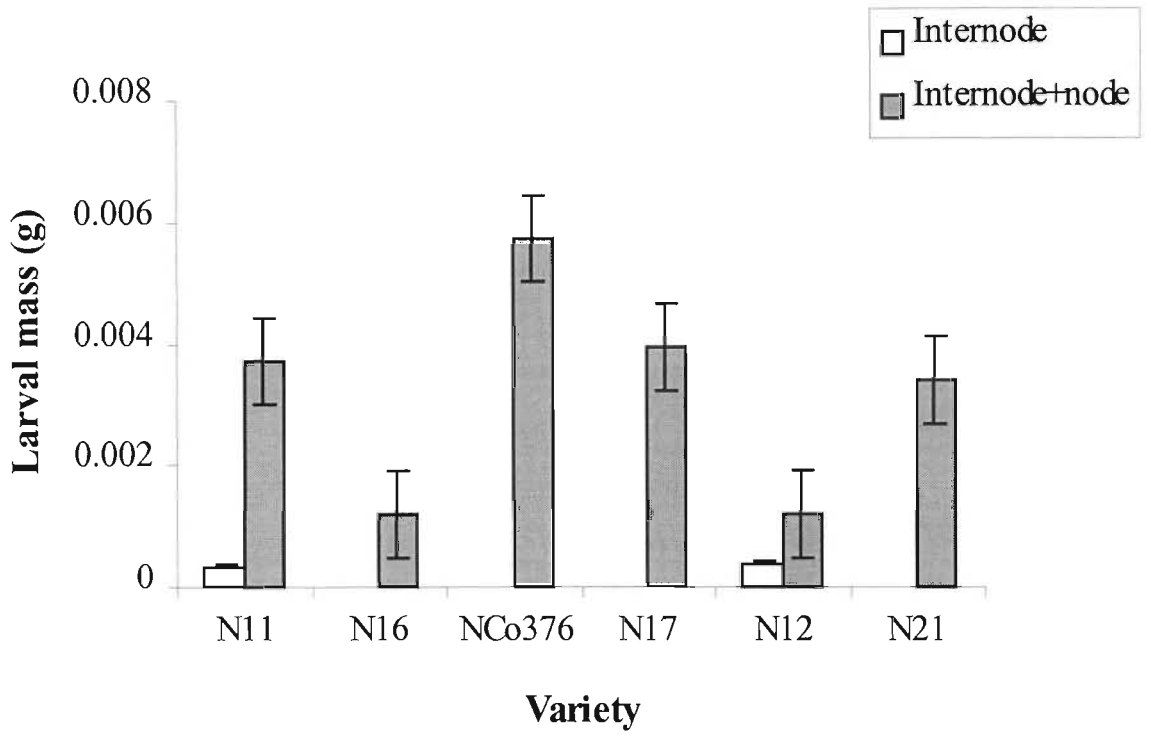

Figure 11: Mean mass (bars = Standard Error) of larvae recovered from internodes & internodes with nodes after 14 days.

CHAPTER 5: SUCCESS OF STALK PENETRATION AND LARVAL GROWTH ASSOCIATED WITH POINT OF ENTRY ON THE NODE

5.1 FEEDING OF NEONATES ON ROOT PRIMORDIA

5.1.1 METHODS

Microcentrifuge tubes were placed on the root primordia on each node. In one instance, wax on the root primordia was removed with dry cotton ('treatment') and in another, the tube was placed over root primordia without manipulation of the area ('control'). One larva was then placed in each tube with a paintbrush. Larvae were left to feed for seven days. At harvest, stalks were dissected and the length of boring and the number of recovered larvae were recorded. Even though there was no survival after seven days, the damage could still be measured.

5.1.2 RESULTS AND DISCUSSION

There were no significant differences in the feeding of larvae on different varieties ($F=1.137$, $P=0.391$) (Fig. 12).

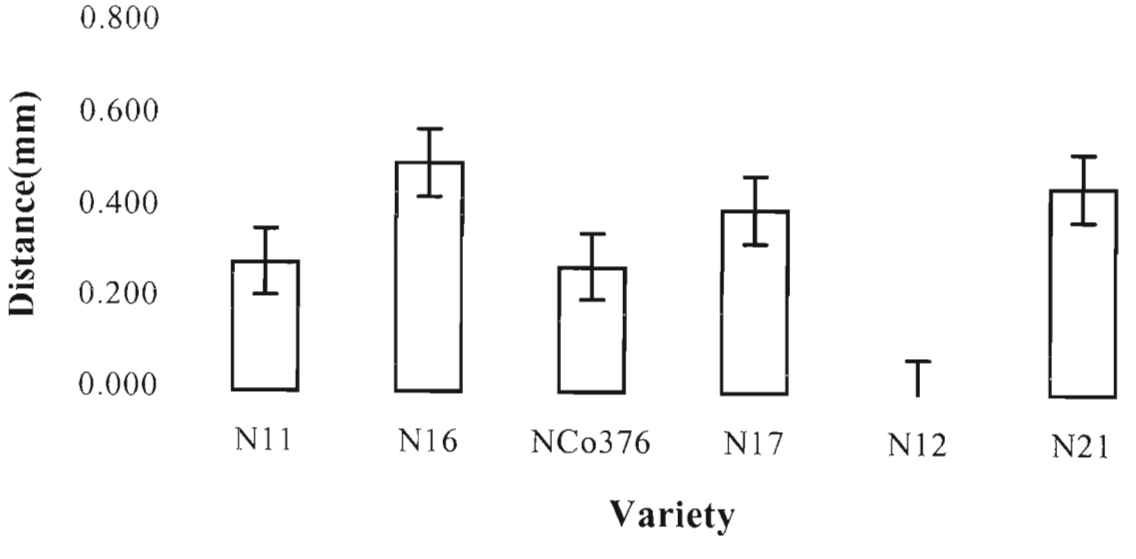

Figure 12: Mean distance covered by larvae (bars = Standard Error) after feeding for seven days.

5.2 FEEDING OF NEONATES ON THE BUD

For each of the following experiments, microcentrifuge tubes were placed over each bud and one neonate was placed inside and left to feed for 14 days.

5.2.1 BUD REMOVED ('TREATMENT') AND BUD + BUDSCALE INTACT ('CONTROL')

The entire bud was carefully removed with a surgical blade to leave a scar on one node ('treatment') and left intact with its scale on the other ('control'). If the larvae fed on the 'control' are larger than the ones fed on the 'treatment', that would mean that the bud is a nutritious source of food for neonates even though they would still have to bore into the budscale first. If the masses of larvae are higher on a susceptible variety than on a resistant one under the same treatment, then larvae on the resistant variety feed because they are not given a choice, not because they would prefer the variety under normal conditions.

5.2.1.1 RESULTS AND DISCUSSION (LABORATORY)

'Treatment' (bud removed): There were significant differences in the masses of larvae among varieties ($F=15.803$, $P<0.001$) (Fig. 14). Larvae that fed on NCo376 were the largest and those that fed on N12, the smallest. The survival numbers were high in all varieties, with the minimum of 83% (Fig. 13).

'Control' (bud + budscale intact): There were significant differences in the masses of larvae among varieties ($F=2.966$, $P=0.038$) (Fig. 14), with NCo376 yielding the largest larvae and N21 the smallest. NCo376 had 100% survival with N12 having the lowest survival (33%) (Fig. 13). The results show that the softness of a variety enhances feeding and penetration of the stalk, irrespective of whether the bud is present or not.

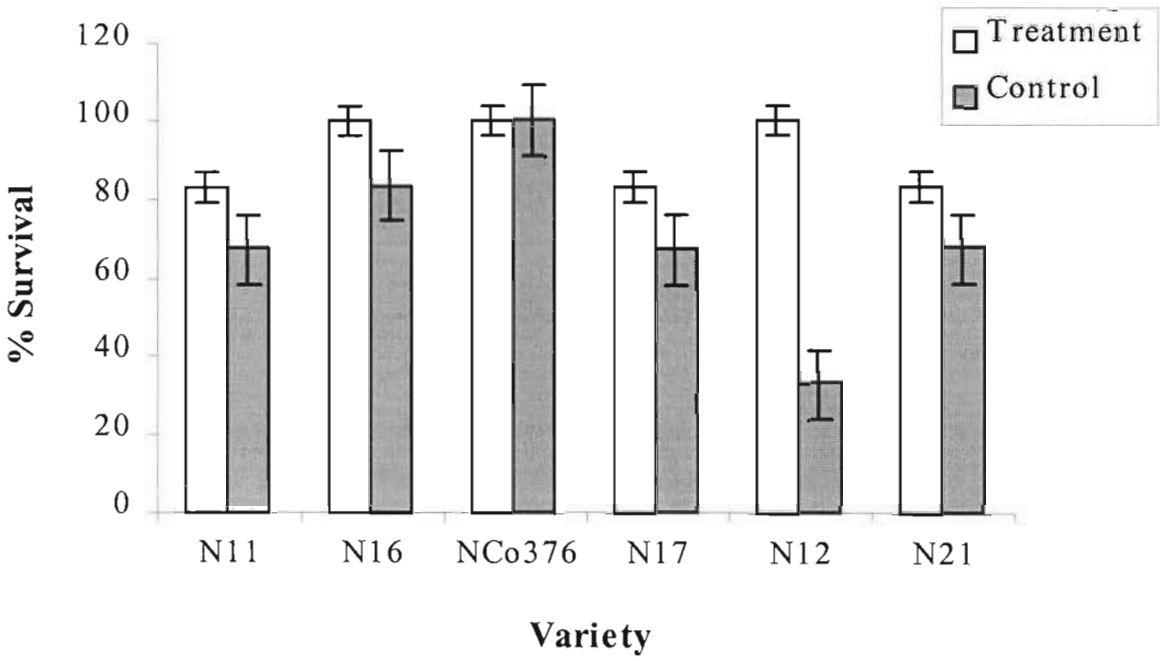

Figure 13: Percentage survival of larvae (bars = Standard Error) recovered from 'bud removed' and 'bud + budscale intact' in the laboratory after feeding for seven days.

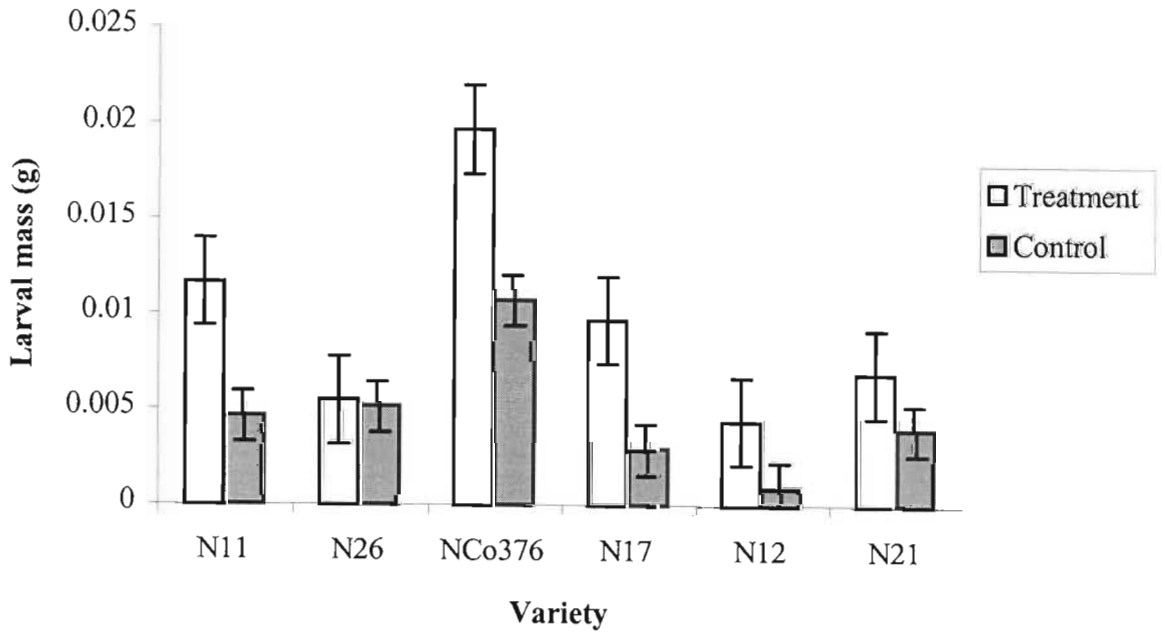

Figure 14: Mean mass gained by larvae after feeding on the 'bud removed' and 'bud + budscale intact' in the laboratory for seven days.

5.2.1.2 RESULTS AND DISCUSSION (GLASSHOUSE)

'Treatment' (bud removed): There were significant differences in the masses of larvae among varieties ($F=15.803$, $P<0.001$) (Fig. 16). All varieties had high survival numbers, with a minimum of 83% (Fig. 15).

'Control' (bud + budscale intact): There were no significant differences in the masses of larvae among varieties ($F=2.260$, $P=0.090$) (Fig. 16). N12 and N17 had the lowest survival numbers (33%) and NCo376 and N21 the highest (100%) (Fig. 15).

The 'treatments' in both the laboratory and the glasshouse yielded similar results, but the 'controls' did not.

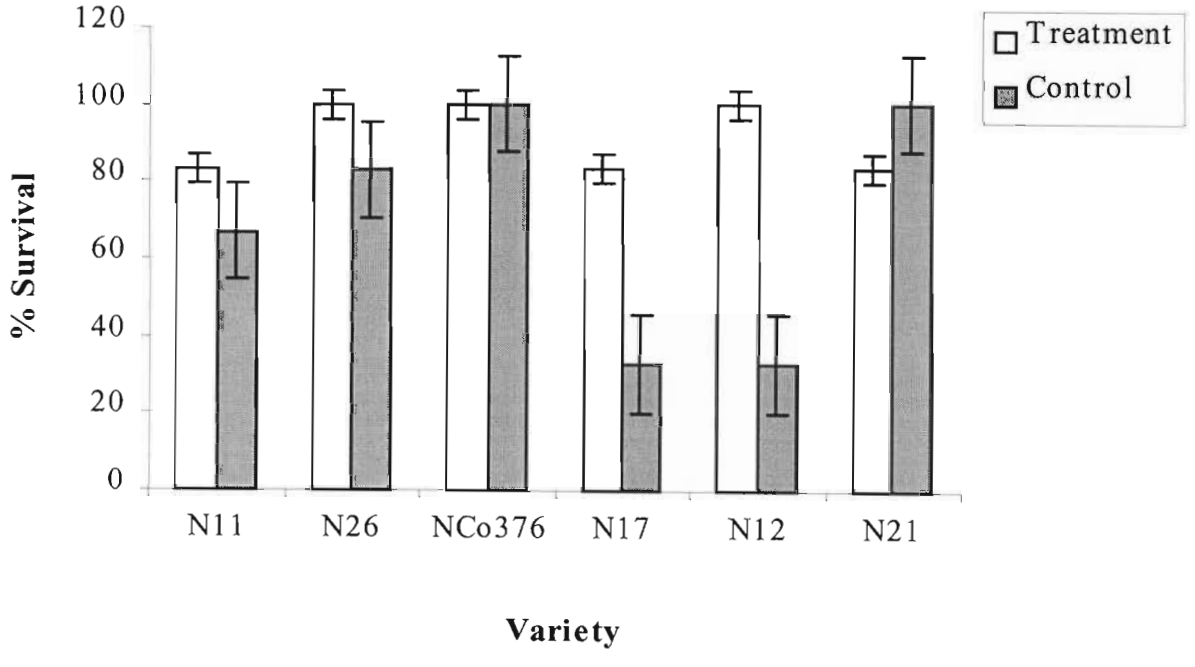

Figure 15: Percentage survival of larvae (bars = Standard Error) recovered from 'bud removed' and 'bud + budscale intact' in the glasshouse after feeding for seven days.

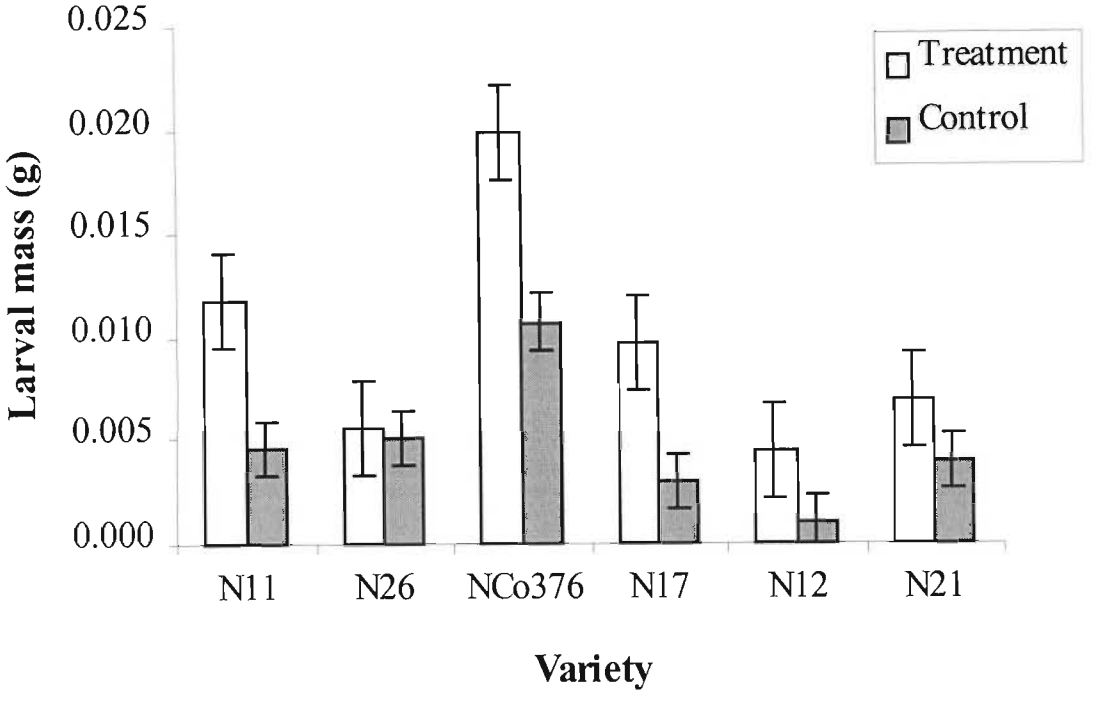

Figure 16: Mean mass gained by larvae after feeding on the 'bud removed' and 'bud + budscale intact' in the glasshouse for seven days.

5.2.2 BUDSCALE REMOVED ('TREATMENT') AND BUDSCALE INTACT ('CONTROL')

The budscale was removed with a surgical blade in one node ('treatment') of each stalk segment and left intact in the other node ('control'). In this way, larval feeding could be compared on stalks with the budscale and on those without the budscale. If larvae penetrated the stalks without the budscale more and faster, then their masses would be more than the ones fed on the bud with a budscale. Because they would not have to feed on the budscale before they get into the bud and their chances of establishment would be increased.

5.2.2.1 RESULTS AND DISCUSSION (LABORATORY)

'Treatment' (budscale removed): There were significant differences in the masses of larvae among varieties (F=2.702, P=0.040) (Fig. 18). N21 had the lowest survival (83%) with the rest of the varieties obtaining 100%. This indicates that the budscale plays a role in impeding larval penetration.

'Control' (bud + budscale intact): There were no significant differences (H=10.501, P=0.062) (Fig. 18), which suggests that larvae trying to penetrate different varieties are affected equally by the budscale. The budscale may therefore play a role in blocking the entry of larvae in both susceptible and resistant varieties. Even though differences were not significant, NCo376 still yielded larvae with the largest masses and N21, the lowest. Larval survival was inconsistent with the known varietal resistance ratings, because N12 yielded the lowest number of larvae (33%), followed by N11 and N21. N17 had the highest number of survivors at 83% (Fig. 17).

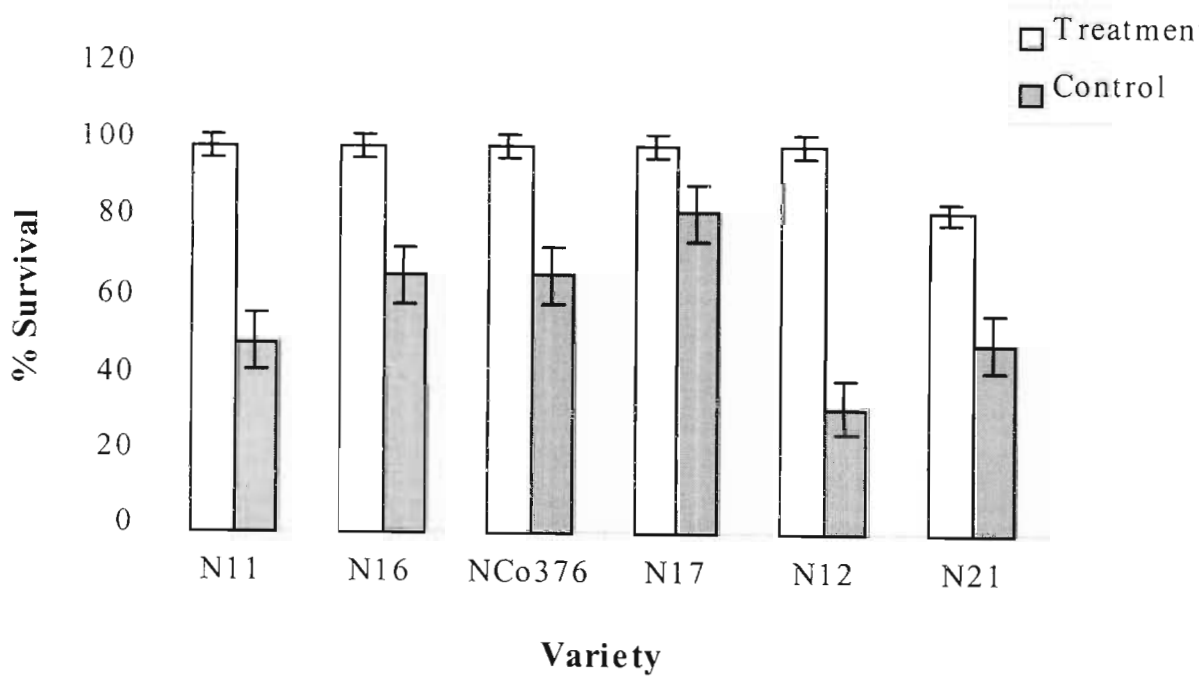

Figure 17: Percentage survival of larvae (bars = Standard Error) recovered from 'budscale removed' and 'bud + budscale intact' in the laboratory after feeding for seven days.

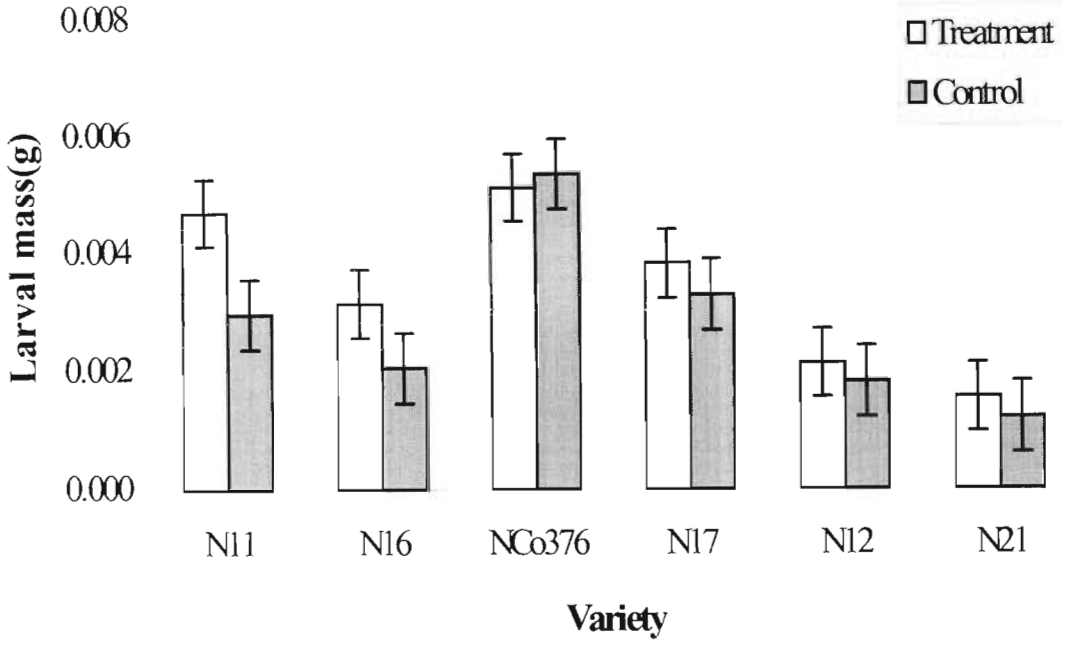

Figure 18: Mean mass gained by larvae after feeding on the 'budscale removed' and 'bud + budscale intact' in the laboratory for seven days.

5.2.2.2 RESULTS AND DISCUSSION (GLASSHOUSE)

'Treatment' (budscale removed): There were significant differences in the masses of larvae among varieties (H=16.236, P=0.006) (Fig. 20). All varieties had high numbers of survivors with the lowest having 83% (Fig. 19).

'Control' (budscale intact): There were no significant differences in the masses of larvae (F=1.502, P=0.280) (Fig 20). Survival numbers were not consistent with the known resistance ratings, NCo376 and N17 (intermediate varieties) yielded the highest survival (67%) and the rest of the varieties, 33% (Fig. 19).

The results were similar in both the laboratory and the glasshouse.

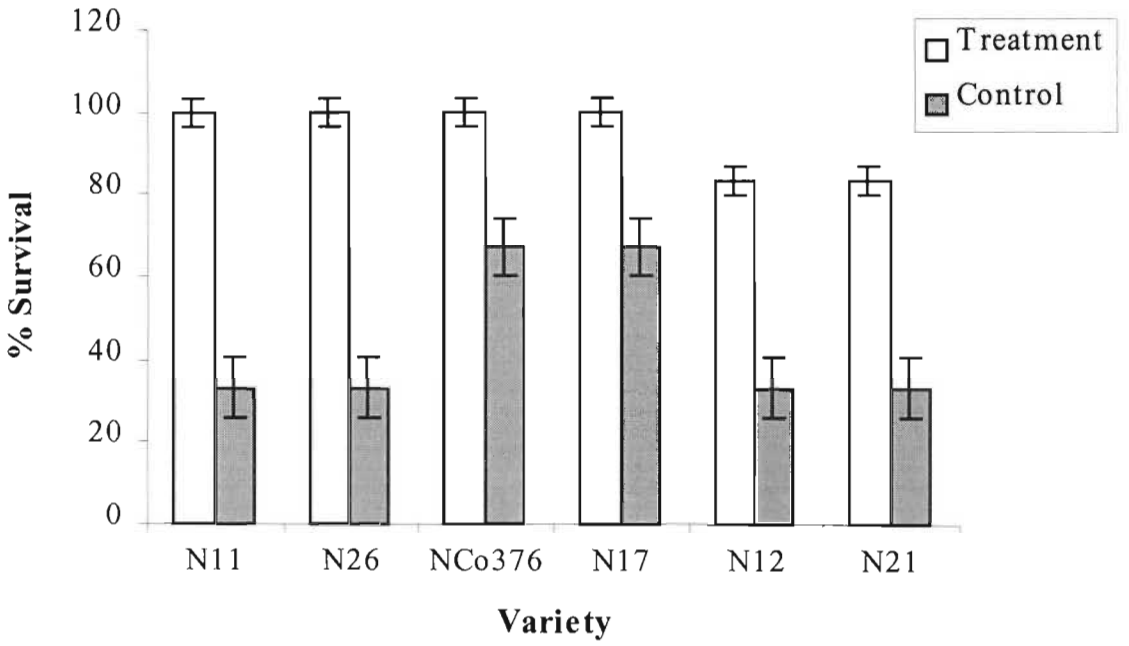

Figure 19: Percentage survival of larvae (bars = Standard Error) recovered from 'budscale removed' and 'bud + budscale intact' in the glasshouse after feeding for seven days.

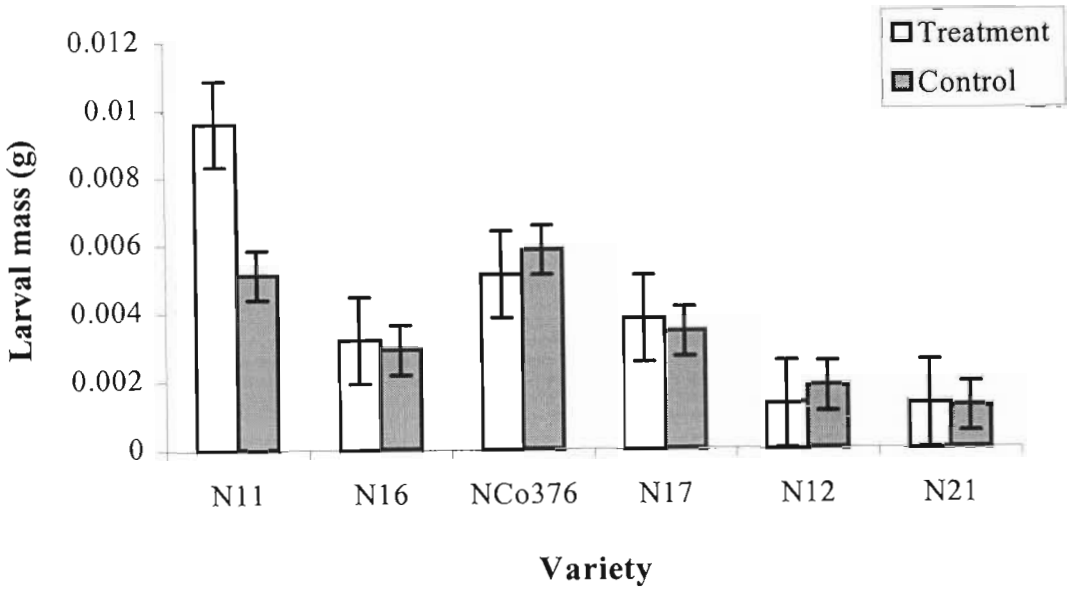

Figure 20: Mean mass gained by larvae after feeding on the 'budscale removed' and 'bud + budscale intact' in the glasshouse for seven days.

5.2.3 BUD REMOVED ('TREATMENT') AND BUDSCALE REMOVED ('CONTROL')

The bud was removed entirely ('treatment') on one node and only the budscale was removed from the bud on the other node ('control'). This was done to test if the bud is of any nutritious value to newly emerged larvae, if this is so, then larvae fed on the 'control' would be larger.

5.2.3.1 RESULTS AND DISCUSSION (LABORATORY)

'Treatment' (bud removed): There were significant differences in masses of larvae among varieties ($F=6.361$, $P=0.003$) (Fig 22). N12 had the lowest survival (33%) and N11, N16 and N17 the highest (67%) (Fig. 21).

'Control' (budscale removed): There were no significant differences ($F=1.554$, $P=0.260$) (Fig. 22). Although non-significant, the differences show very similar trends to those found on the previous experiments, that is, susceptible varieties yield larvae with large masses as opposed to resistant varieties. NCo376 had the lowest number of survivors at 16% and N12 the most at 83%

(Fig. 21). This result was inconsistent with any of the above experiments. The results also mean that as long as the bud is exposed, larvae will feed, irrespective of which variety it is that they are confined to, problems may arise once larvae penetrate the stalk.

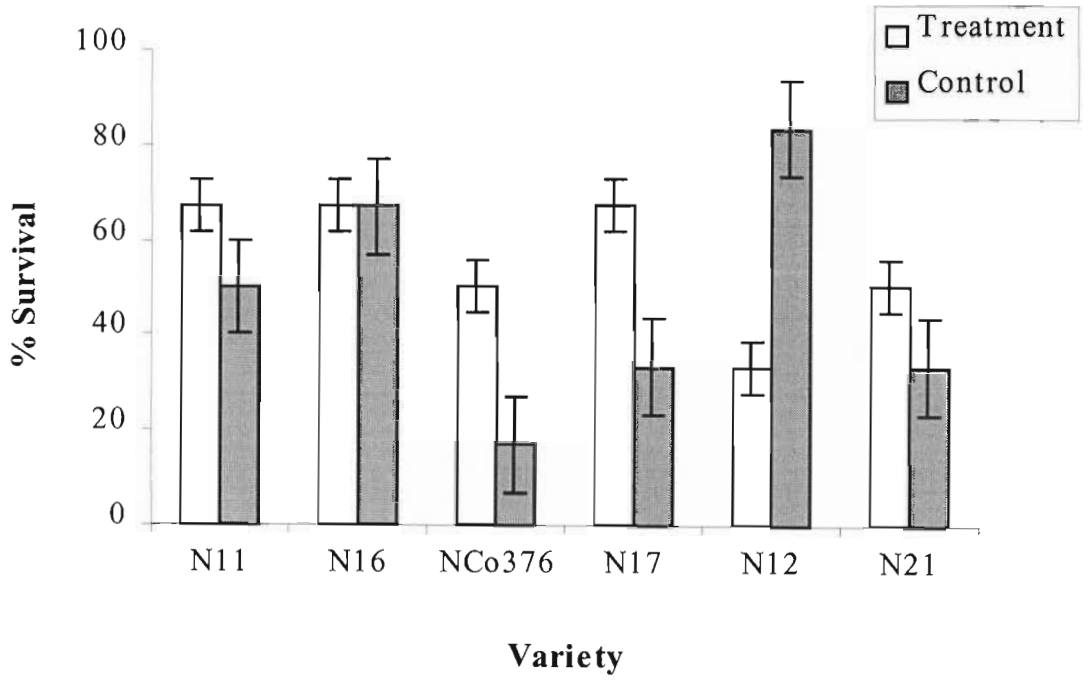

Figure 21: Percentage survival of larvae (bars = Standard Error) recovered from 'bud removed' and 'budscale removed' in the laboratory after feeding for seven days.

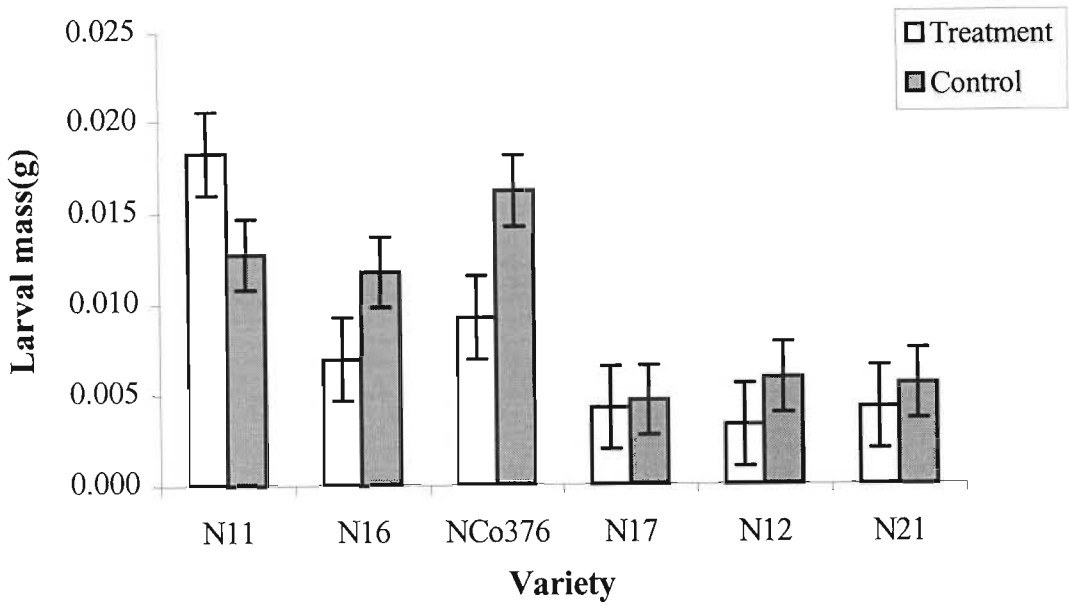

Figure 22: Mean mass gained by larvae after feeding on the 'bud removed' and 'budscale removed' in the laboratory for seven days.

5.2.3.2 RESULTS AND DISCUSSION (GLASSHOUSE)

'Treatment' (bud removed): There were significant differences in the masses of larvae ($F=2.873$, $P=0.049$) (Fig. 24). N12 had the lowest number of survivors at 33% and N26 the highest at 83% (Fig. 23).

'Control' (budscale removed): There were significant differences in the masses of larvae ($F=2.702$, $P=0.040$) (Fig. 24). N26 had the lowest number of survivors (83%) and the rest of the varieties had 100% survival.

The 'treatment' in the laboratory yielded similar results to that in the glasshouse, but the control in the glasshouse showed that larval feeding differs with varieties, that is the masses differ even though the survival was high in all varieties.

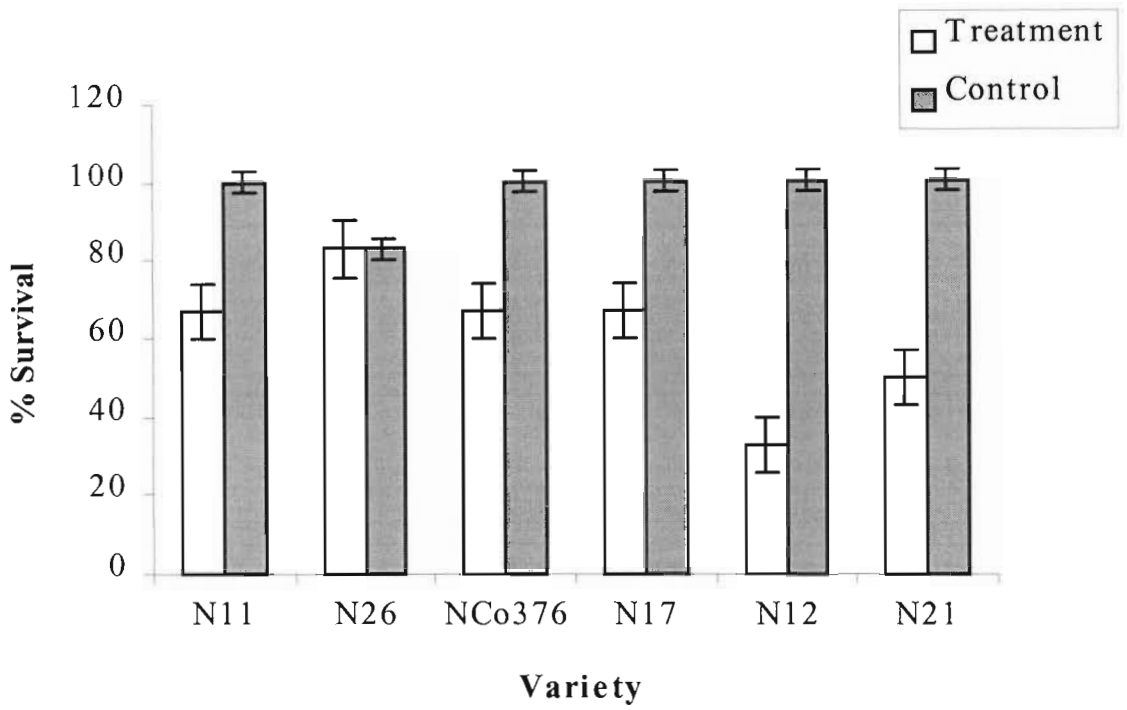

Figure 23: Percentage survival of larvae (bars = Standard Error) recovered from 'bud removed' and 'budscale removed' in the glasshouse after feeding for seven days.

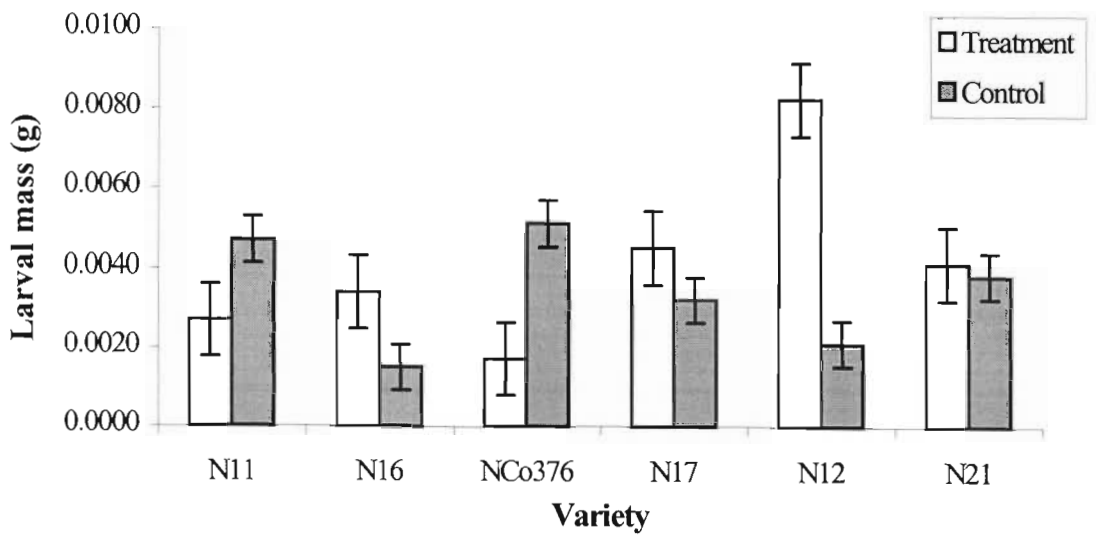

Figure 24: Mean mass gained by larvae after feeding on the 'bud removed' and 'budscale removed' in the glasshouse for seven days.

Rutherford (1998) reported that chlorogenates and flavonoids appear to be involved with resistance and/or susceptibility to *E. saccharina* in sugarcane. The initial invasion of sugarcane by *E. saccharina* from neighbouring *Cyperus papyrus* swamps in northern KwaZulu-Natal (Atkinson *et al.*, 1981) may have been facilitated by flavonoid similarities between these plant families (Rutherford, 1998). There is a correlation between increasing flavonoid content and susceptibility of *E. saccharina*. In contrast, maize lines accumulating greater amounts of the flavonoid maysin are more resistant to the corn earworm, *Helicoverpa zea* (Wiseman *et al.*, 1992). Flavonoids are involved in many plant-insect interactions and can be active in host-plant recognition, feeding stimulation or deterrency, as well as having effects on insect physiology and nutrition (Slansky, 1992).

5.3 RIND HARDNESS

5.3.1 USING A PENETROMETER

5.3.1.1 INTRODUCTION

Dillewijn (1952) traced the development of instrumentation for testing the rind hardness of sugarcane to Puri & Vankatram (1929), Hedley (1936), and Pemberton (1936). Investigations since then have reported variations in rind hardness due to both environmental and genetic factors. Although environmental factors have been shown to affect hardness (Martin & Cochran, 1975), Hedley (1936) reported that, within one field, all stalks showed uniform trends. Internodes from the top of the stalk are generally softer than those from near the bottom (Martin & Cochran, 1975).

5.3.1.2 METHODS

Measurements were taken with a penetrometer from the same varieties used for rind hardness experiments to compare results with those obtained when using larvae. Six stalks of each variety were punctured with a penetrometer at two different parts and the reading was taken.

5.3.1.3 RESULTS AND DISCUSSION

This small experiment showed no significant differences among the used varieties. N11 was the softest, followed by N16, N12 and N21 (Fig. 25).

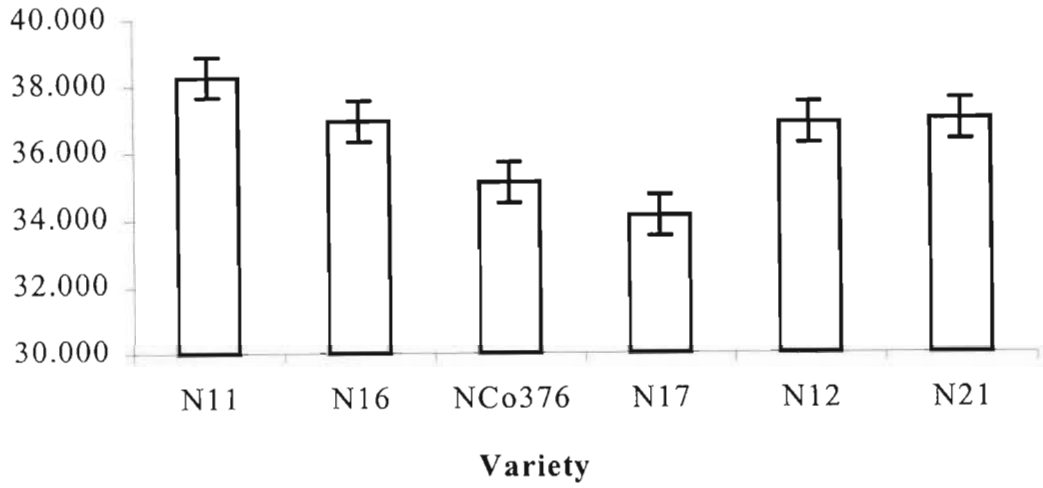

Figure 25: Mean 'hardness' values, with bars representing Standard Error.

Martin & Cochran (1975) tested sugarcane internode rind hardness in NCo310, L 60-25 and L 62-96 (Louisiana varieties) using *Diatraea saccharalis* and they observed that varietal differences exist, not only in maximum hardness, but also that varietal rank with respect to hardness varies with position along the stalk. Thus, varietal differences in development of internode hardness must exist. They found significant differences in hardness among varieties at the third internode that represented varietal differences in initial rind hardening. The varietal rank with respect to hardness at the bottom of the third internode was identical to the rank with respect to varietal borer resistance. This information, along with the fact that internodes attacked by borers are harder than undamaged internodes of comparable stalk position and that maximum thresholds in borer damage do exist, suggested that differences in resistance to borer attack among sugarcane varieties may be associated with internode hardness in the top of the stalk.

At the bottom of the sixth internode, however, no difference in rind hardness was detected among the three varieties used (NCo310, L 62-96 and L 60-25) at the 5% significance level. Regardless of the number of internodes per stalk, the hardness of a given internode, numbered relative to the apex, is consistent among stalks within varieties (Martin & Cochran, 1975).

A rind hardness development gradient exists in the cane from top downwards and varietal rank with regard to rind hardness was found to be dependent upon the maturity of the internode measured (Martin & Cochran, 1975).

5.3.2 LARVAL FEEDING ON THE RIND

5.3.2.1 GENERAL METHODS

Thirty-six stalk segments were placed horizontally in the cage in a randomised complete block. Damaged internodes were not used. Each stalk segment was considered a replicate. Microcentrifuge tubes were placed on wax bands with the rind and wax removed (1 mm deep) with a cork-borer ('treatment') and rind and wax intact ('control'). The internode was tested for larval penetration in 'screening experiments' and the results showed that it is not a favourable penetration points for larvae in any of the varieties used, thus, the use of the rind on the node for 'larval feeding experiments'. One larva was placed in each tube. For neonates, the larva was left for 14 days to allow time for feeding to a measurable weight, while second and third-instar larvae were only left to feed for seven days due to their fast feeding rate, thus becoming to large for the tubes. Larval feeding behaviour was observed every two days. Since larvae were weighed before and after feeding, analysis was done on the difference (gained/lost mass).

5.3.2.2 USING NEONATE LARVAE

5.3.2.2.1 RESULTS AND DISCUSSION (LABORATORY AND GLASSHOUSE)

All larvae died in both the 'treatment' and the 'control'. Carnegie (1974) mentioned that larvae feed on cane leaves, or else as scavengers on organic matter for about a week before penetrating the stalk. One way of interpreting this result is that larvae starved to death, because they did not have hardened mandibles to feed on the rind or inside the stalk.

5.3.2.3 USING SECOND-INSTAR LARVAE

5.3.2.3.1 RESULTS AND DISCUSSION (LABORATORY)

All second-instar larvae on the 'treatment' survived and only larvae on N12 lost weight (33%). In the 'treatment' there were significant differences among varieties ($F=4.186$, $P=0.005$). However, the trends were not as expected for most of the varieties, except for N12 which showed high resistance (69% survival).

In the 'control', none of the larvae survived, indicating that second-instar larvae also cannot penetrate on the wax band when the rind is intact. Figure 26 shows the differences in the gained mass in each variety.

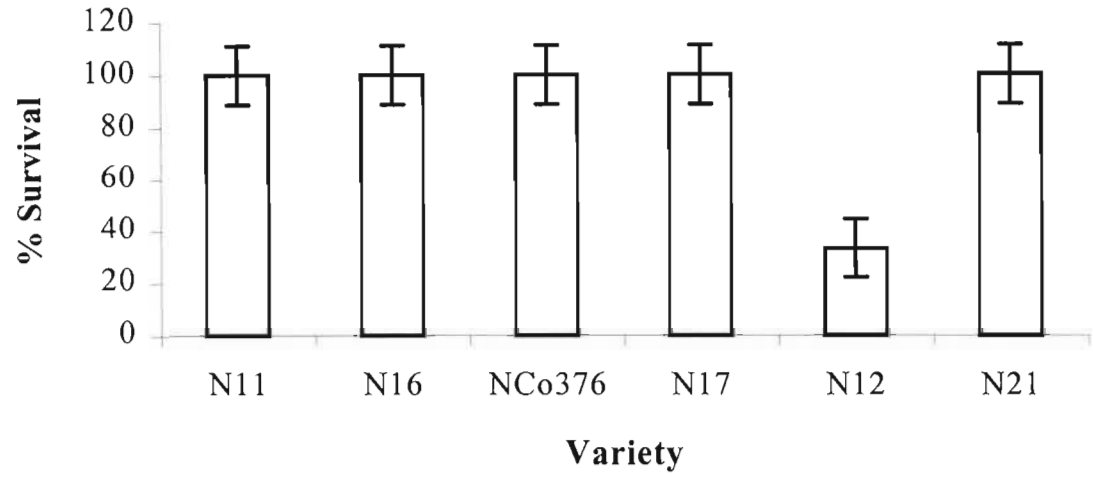

Figure 26: Percentage survival of second-instar larvae in the laboratory (bars = Standard Error) after feeding on 'rind removed' ('treatment') after feeding for seven days.

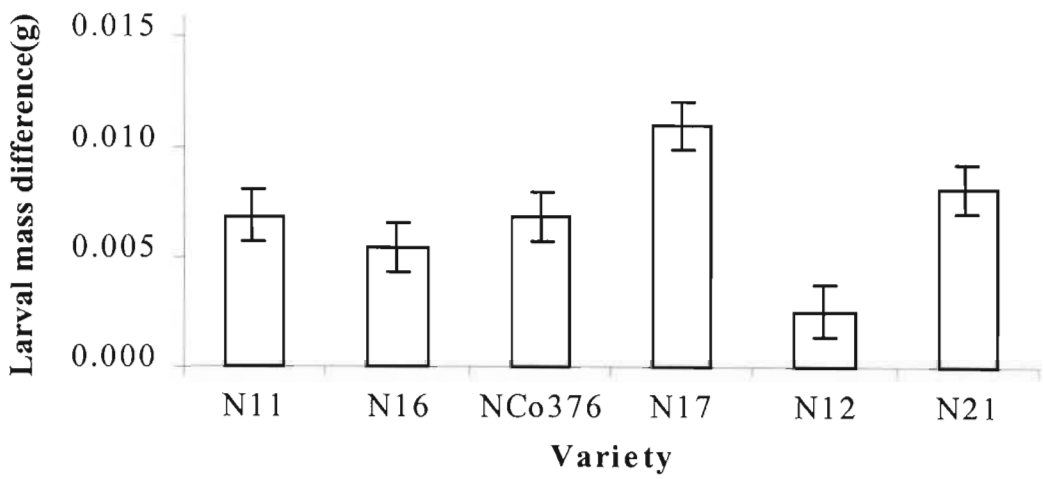

Figure 27: Mean mass gained by second-instar larvae in the laboratory (bars = Standard Error) after feeding on 'rind removed' ('treatment') for seven days.

5.3.2.3.2 RESULTS AND DISCUSSION (GLASSHOUSE)

N17 and N21 had no survivors on the 'treatment' (rind removed). Live stalks secrete 'juice' when damaged and larvae might have drowned from this. N11 had large larvae on the 'treatment' followed by NCo376. There were no significant differences in the masses of larvae (F=2.024, P=0.164).

'Control': There were no significant differences (F=0.343, P=0.876). N26 had the highest number of survivors at 67% and N11, N12 and N17 the lowest at 33%.

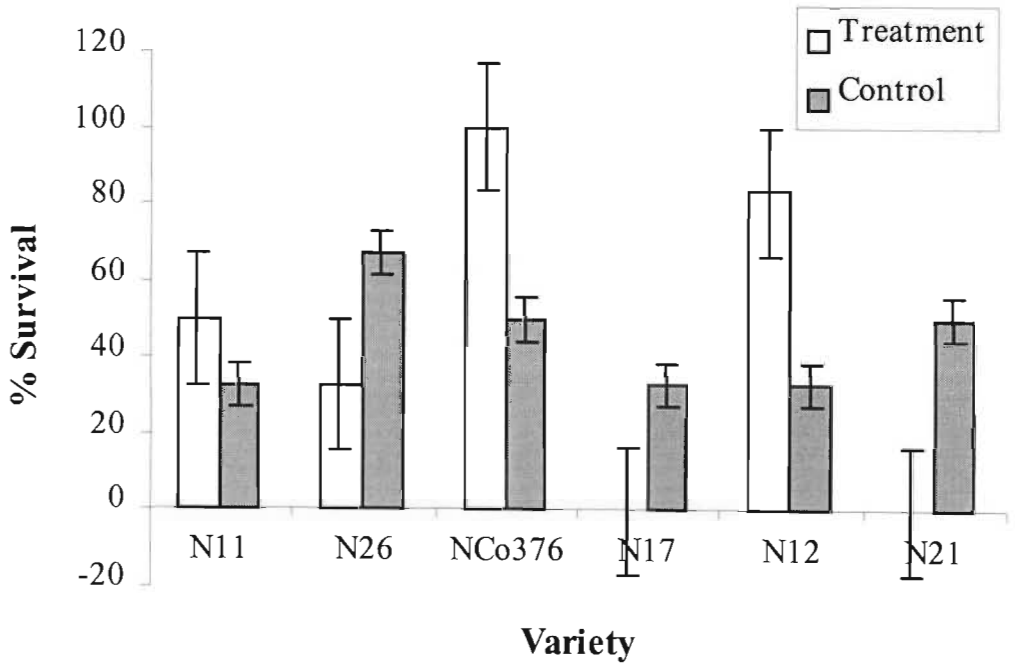

Figure 28: Percentage survival of second-instar larvae in the glasshouse (bars = Standard Error) after feeding on 'rind removed' ('treatment') for seven days.

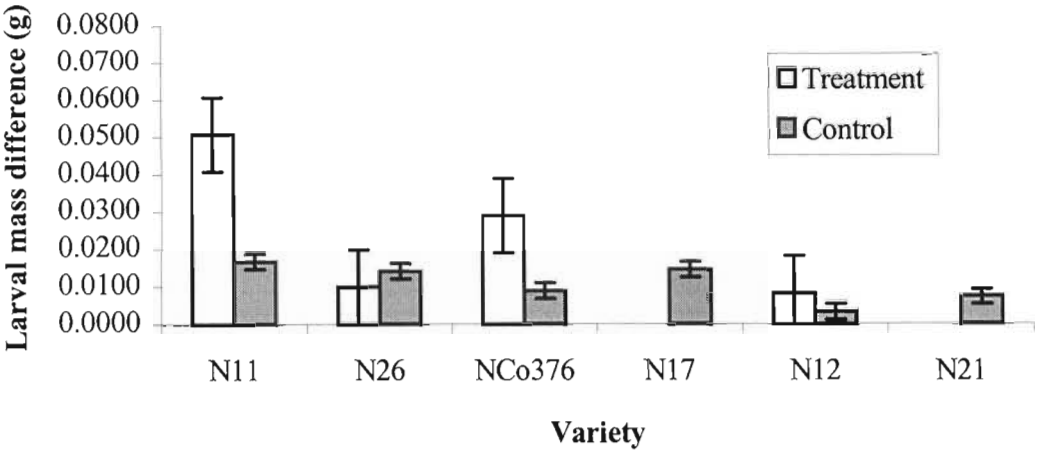

Figure 29: Mean mass gained by second-instar larvae in the glasshouse (bars = Standard Error) after feeding on the 'rind removed' ('treatment') and 'rind intact' ('control') for seven days.

5.3.2.4 USING THIRD-INSTAR LARVAE

5.3.2.4.1 RESULTS AND DISCUSSION (LABORATORY)

There were no significant differences in larval masses among varieties in both 'treatment' ($F=0.342$, $P=0.883$) and 'control' ($H=6.379$, $P=0.271$) and the patterns were difficult to interpret.

There was also no significant difference between the 'treatment' and 'control', meaning that larvae fed with equal ease when the rind was removed or intact. In some cases, larvae did not bore in at all, but were still alive after seven days without feeding (Figure 31).

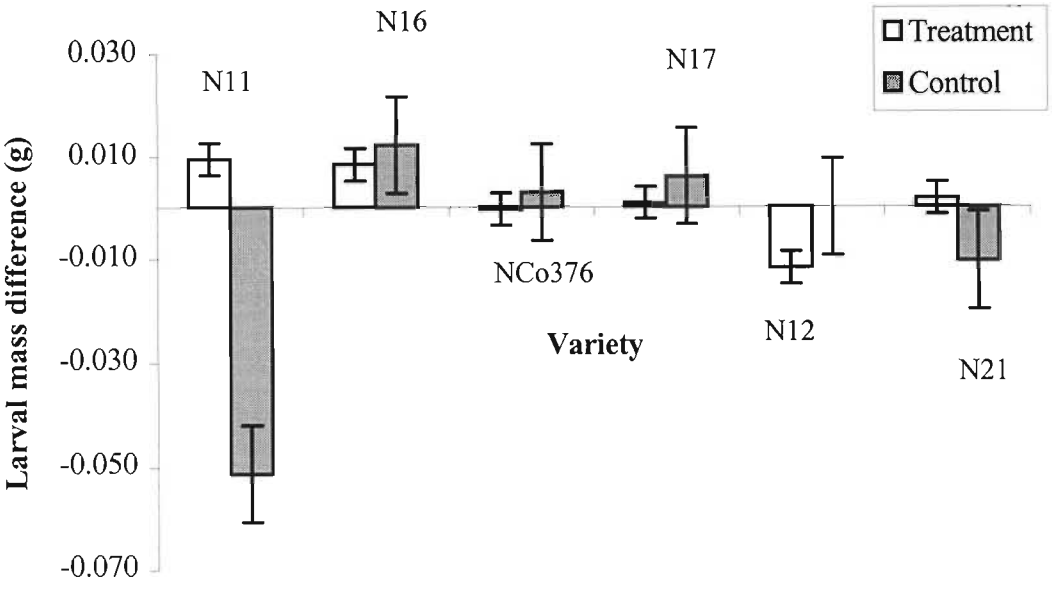

Figure 30: Mean mass gained by third-instar larvae (bars = Standard Error) after feeding on 'treatment' (rind removed) and 'control' (rind intact) for seven days in the laboratory.

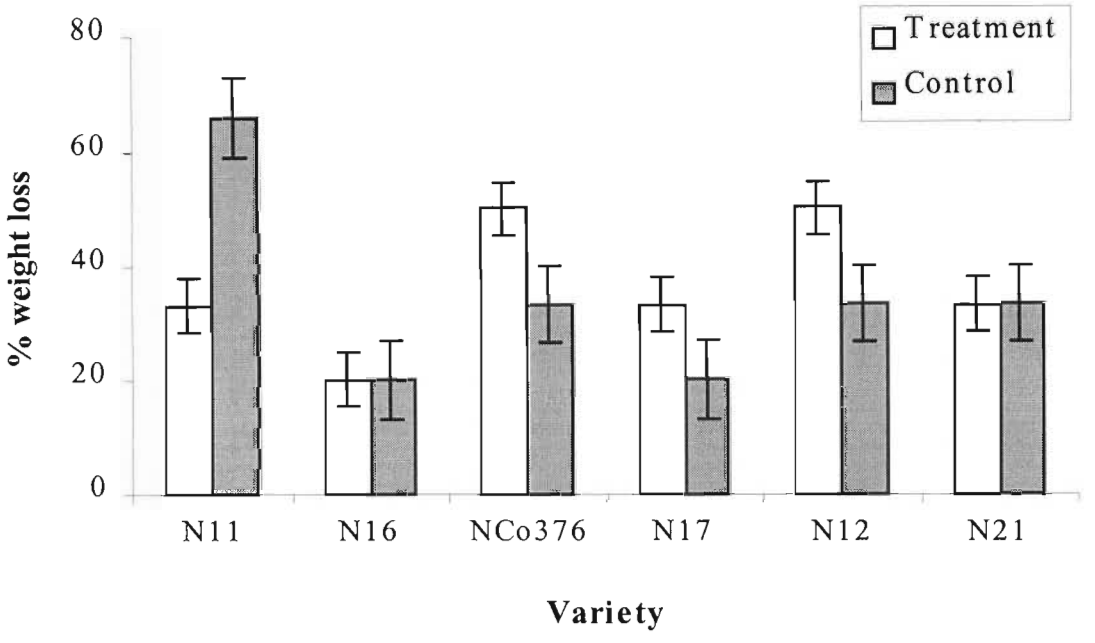

Figure 31: Percentage number of larvae that lost weight (larvae that did not feed) on the 'rind removed' ('treatment') and 'rind intact' ('control') after seven days in the laboratory.

5.3.2.4.2 RESULTS AND DISCUSSION (GLASSHOUSE)

'Treatment': There were no significant differences (F=2.024, P=0.164). N12 has the highest survival (83%) and N17, the lowest (33%). These results show that large larvae have no difficulty feeding on the rind when not given a choice.

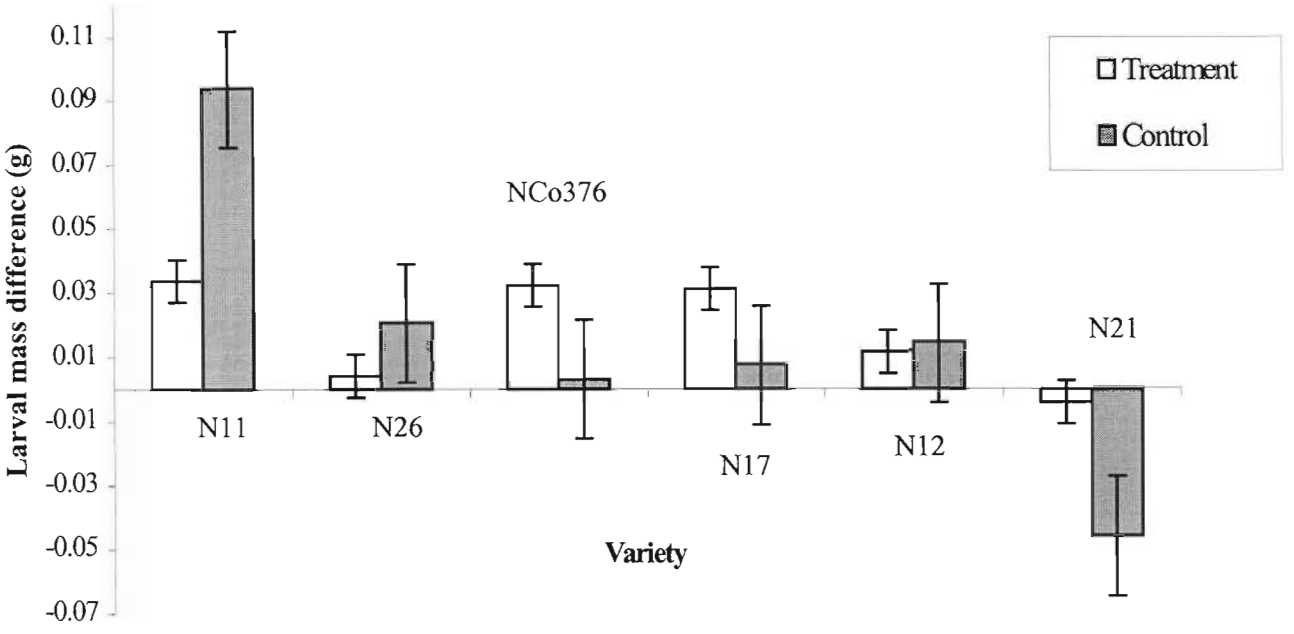

Figure 32: Mean mass gained by third-instar larvae (bars = Standard Error) after feeding on 'treatment' (rind removed) and 'control' (rind intact) for seven days in the glasshouse.

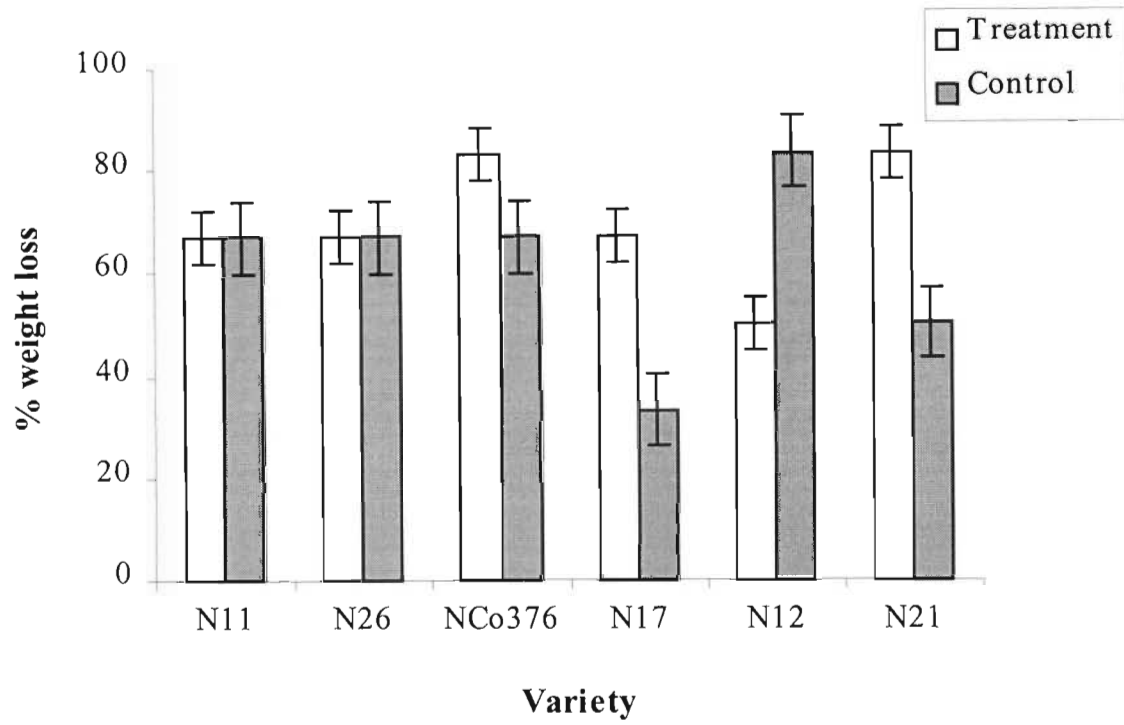

Figure 33: Percentage number of larvae that lost weight (larvae that did not feed) on the 'rind removed' ('treatment') and 'rind intact' ('control') after seven days in the glasshouse.

Studies on the relationship of sugarcane internode hardness to larval damage (Martin *et al.*, 1975) made in eight varieties showed that varietal resistance to *Diatraea saccharalis* was associated with hardness of the target internodes (David & Joseph, 1982). Hardness of the rind has been found to be a major factor in resistance of commercial varieties to *D. saccharalis* infestation (Martin & Cochran, 1975). The varieties that are hard at the target internode and those in which the leaf sheaths envelop the target internodes tightly, successfully prevent attack by neonate larvae (David & Joseph, 1982).

CHAPTER 6: DIET INCORPORATION--ADDING EPICUTICULAR WAXES

6.1 INTRODUCTION

The primary role of epicuticular lipids on aerial plant surfaces is prevention of water loss, but mediation of interactions between plants and insect herbivores is also an important role. The physical structure of plant surface lipids can affect insect herbivore attachment and movement. Surface lipids can also affect insect herbivores indirectly by influencing predatory and parasitic insects (Eigenbrode & Espelie, 1995). The epicuticular waxes of a plant can vary with plant part, age, and environmental conditions (Baker, 1982).

Rutherford & van Staden's (1996) results from a feasibility study suggest that chemical differences exist in sugarcane wax that can be correlated with resistance or susceptibility to *E. saccharina*. The results obtained suggest that variations in wax characteristics might account for around 53.5% of the variation in larval survival ratings. The correlation between predicted and known ratings declined with increasing cane age. Fractionation of waxes from resistant and susceptible varieties suggests that the alcohol fraction decreases as a proportion of the total with both susceptibility and age. The C_{26} aldehyde and alcohol and the C_{28} alcohol were associated with resistance while C_{30} alcohol appeared to be associated with susceptibility. It is therefore possible that the high alcohol/aldehyde ratio and shorter chain length might be involved in resistance to the stalk borer (Rutherford & van Staden (1996).

These results suggest that wax alcohols might be associated with resistance while aldehydes appear associated with susceptibility. The indication that shorter chain length is also associated with resistance suggests that additional resistance might be obtained through further introgression of selected *S. spontaneum* germplasm (Rutherford & van Staden, 1996).

Waxes from different varieties may have varying effects on the growth and survival of *Eldana saccharina* larvae; that is, they may have an antibiotic effect. The diet was made in the SASEX

Insect Rearing Unit using the regular ingredients (Graham & Conlong 1988; Gillespie 1993) and six crude waxes from NCo376, N11, N12, N16, N17 and N21 for diet incorporation bioassays.

6.2 METHODS

The waxes were scraped from the stalks using razors and kept in separate jars at 4°C until used. Crude wax (2.5g) from each variety was added to 2 litres of diet. The diet containing wax from each variety was dispensed into six multicell trays (for each variety), each tray representing a replicate. After the diet had cooled down and solidified, penetration holes were made with a scarifier and two neonate larvae were placed in each cell using a fine paintbrush. Each cell was covered with corncob grits after inoculation to keep larvae in their cells. The trays were placed in a metal rack and left in a larval growth room for 20 days. Thereafter, the number of surviving larvae in each tray was recorded together with their mass.

6.3 RESULTS AND DISCUSSION

At 20 days, there were significant differences in the collective mass ($F=10.01$, $P<0.001$) and mean mass ($F=16.927$, $P<0.001$) of larvae per tray (Fig. 35). Comparisons between varieties showed that the N21 mix had the largest larvae next to N11. Collective larval mass was significantly lower in the N16 mix than any other mix (0.596g). Larval masses were significantly greater in the N11 mix (1.599g) than in any other mix. The susceptible varieties, N11 and N16 had the lowest number of larval mortality, but there were no significant differences in the survival of larvae amongst varieties.

The results suggest that the differences in wax composition do not necessarily correspond with the known resistance ratings. There might be another factor involved, which decreased survival of larvae on certain varieties.

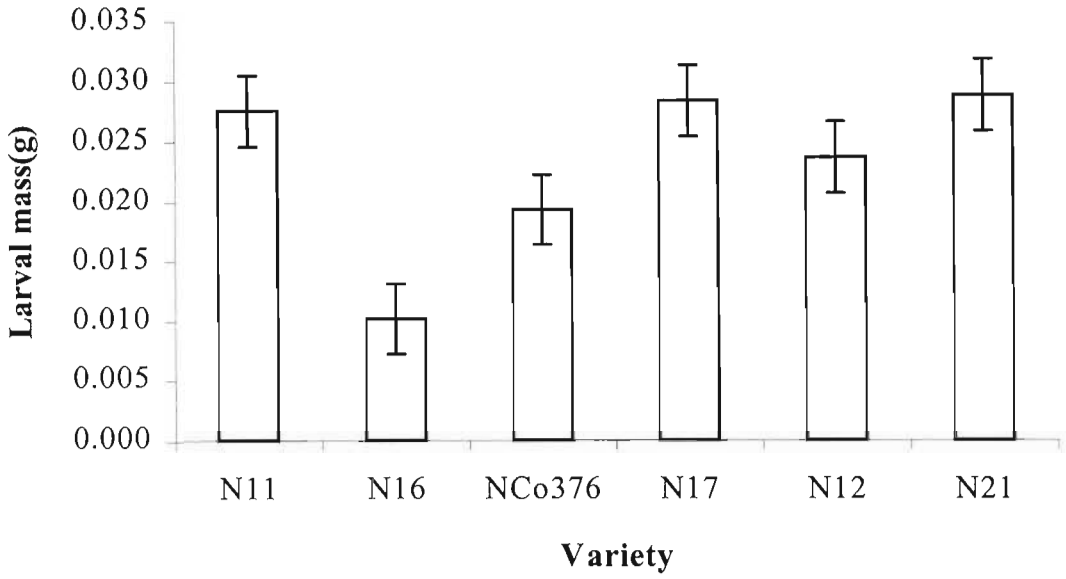

Figure 34: Mean mass per larva (bars = Standard Error) from diets incorporating wax from different varieties.

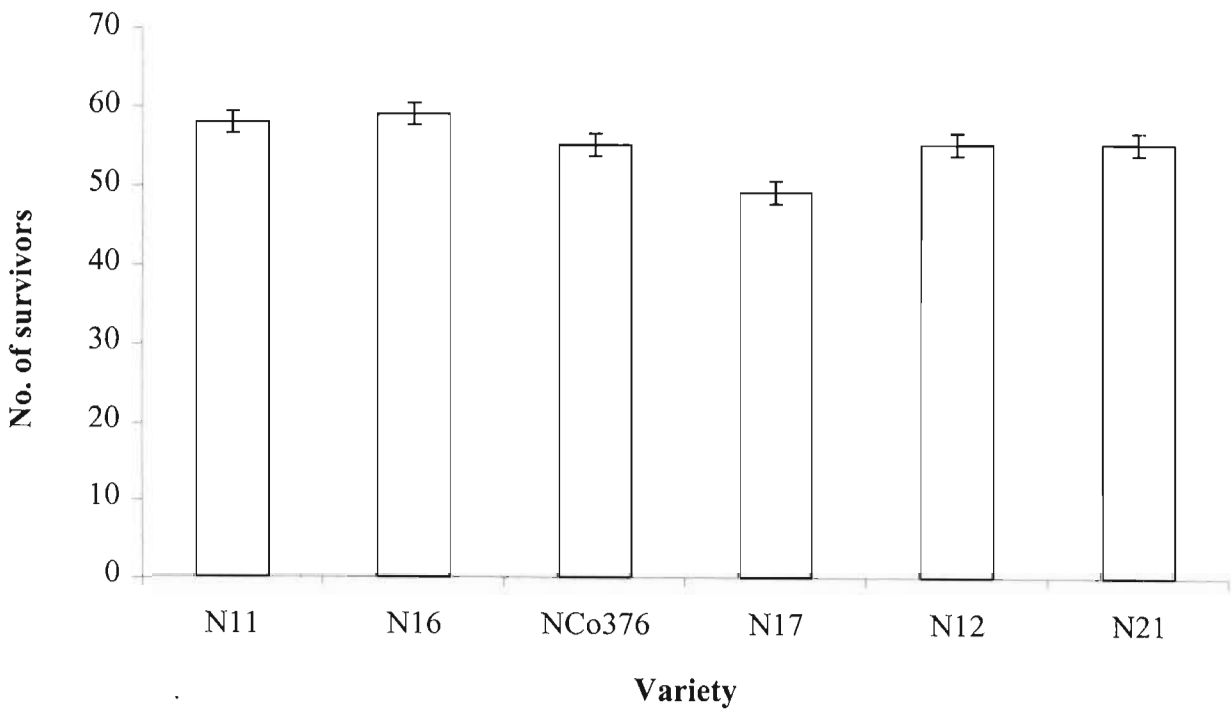

Figure 35: Mean number of surviving larvae on different varieties.

In tests conducted by Woodhead *et al.* (1987), gas chromatographic analysis of surface extracts from three sorghum cultivars showed that there are significant differences in concentration of a compound with a 32 carbon chain length (32 C marker), which could account for observed differences in behaviour when stem borer larvae crawl over the surface of these plants, or over surface extracts in bioassays (Woodhead *et al.*, 1987). The absence of a sufficiently high concentration of this chemical from the plant wax disorients *Chilo partellus* (Woodhead & Taneja, 1987).

CHAPTER 7: MOBILITY OF NEWLY HATCHED LARVAE ON MATURE SUGARCANE PLANTS

7.1 INTRODUCTION

Plants have developed various mechanisms of defence against phytophagous insects. Two defensive morphological features are trichomes and glands. Trichomes are either glandular or non-glandular. Trichomes act as physical barriers keeping smaller insects away from the leaf surface (Stipanovic, 1983). The degree of leaf pubescence greatly affects the behaviour of gravid females of cereal leaf beetle, *Oulema melanopis* (Schillinger & Gallun, 1968). Kyle & Hensley (1970) conducted studies comparing the establishment and damage of sugarcane borer on two sugarcane cultivars. Their studies suggested that the resistance of NCo310 (compared with the susceptibility of 'CP 44-101') to sugarcane borer was due primarily to higher mortality of larvae, especially of young larvae before tunnelling into the internodes. In a later study, Coburn & Hensley (1972) reported that the resistance in NCo310 was due to the occurrence of a tight leaf-sheath that inhibited establishment of larvae. There have been several studies on the general behaviour of sugarcane borer on sugarcane. Information from such studies would be helpful in further understanding mechanisms of resistance (White, 1993).

Useful data on characters that may provide resistance to borers have been variable and inconclusive. Pubescence, or leaf hairs on the lamina, has been associated with pest resistance in sugarcane. Pubescence has been known to interfere with oviposition, attachment of eggs to plant surfaces, feeding, and ingestion of many insects (Maxwell & Jennings, 1980). Webster (1975) reported resistance due to hairiness in 17 crops against 32 insect pests. However, pubescence does not always result in resistance in sugarcane (Sosa, 1988).

Veins in sugarcane leaves are prominent and run parallel to the midrib along the length of the lamina, dividing the leaf surface into ridges and grooves. On these ridges, some sugarcane

varieties have short bulbous spines (denticules); susceptibility to *Scirpophaga nivella* decreases with increasing numbers of denticules per mm length (Verma & Mathur, 1949).

This chapter concentrates on the dispersal of neonate larvae at different intervals after hatching. This information will provide knowledge of the time spent by larvae on different parts of the stalk before they penetrate, which may be useful for treatment with insecticides as well as indicating the likelihood of larval mortality due to other adverse biotic or abiotic factors, e.g. predation, desiccation or dislodgement from the host plant.

7.2 METHODS

Plants that had been planted in 1999 in the shadehouse were used for this experiment. Six plants from each variety (each representing a replication) were moved to a room with controlled temperature and humidity set at 26^0C and 75 %, respectively. One variety was inoculated with black eggs (close to hatching) at a time, thereby allowing one plant to be harvested every day. Eggs were counted under the microscope and batches of 290 to 320 eggs were prepared because it was difficult to get exactly 300 eggs in a batch. Using forceps a batch of eggs was then placed on the bottom part of the live sugarcane stalk, between the leaf sheath and the stalk.

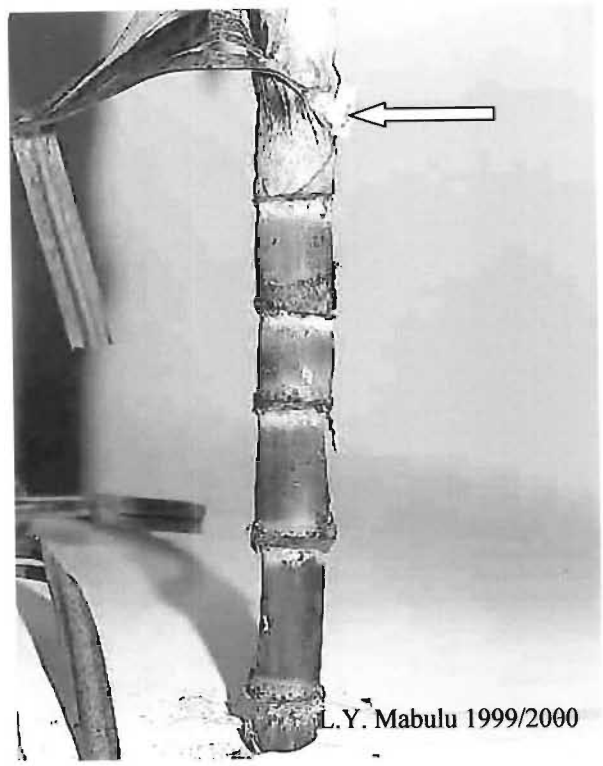

Figure 36: One of the sugarcane stalks used in larval mobility studies. The arrow is pointing at the egg batch on a piece of paper that was placed under the leaf sheath of N11.

Stalks were harvested at 24-hour intervals. Each stalk was systematically searched for larvae. The procedure was as follows: 1) leaf blades with sheaths intact were removed from the stalks and examined, 2) unexpanded leaves of the whorl were removed and carefully unrolled, 3) stalks were examined for entry holes, 4) stalks containing entry holes were split and searched for larvae, 5) leaf scars were carefully shaved to expose larvae that might be unexposed.

7.3 RESULTS AND DISCUSSION

OBSERVATIONS

24 hours after hatching

- Larvae were recovered either on the leaves or on the stalk surface, but none were hidden in leaf scars.
- 22% of the total number of emerged larvae on N12 were recovered at this time interval, followed by 12% on N21. These varieties also had the highest number of larvae trapped in glue, 43 & 78%, respectively. NCo376 had 3% and N11, 7.8%, but no larvae were found on N17 and N26. The larvae were observed moving on the abaxial (upper) surface of green leaf sheaths of the middle segments.

48 hours

- NCo376 had the highest number of larvae (29%), concealed in leaf scars on the bottom segments, followed by N12 with 9%.
- Larvae found on the adaxial (bottom) side of the leaf sheaths were either feeding on soft cracks or on debris at the bottom of the leaf sheaths.
- Those that were recovered on the root bands were observed to feed on the sugary secretions and in soft cracks.
- N12 & 21 still had more larvae trapped in glue, 87 and 34%, respectively.

72 hours

- The dry root primordia had no larvae feeding on them, as opposed to the ones with sugary secretions.
- Larvae recovered from different parts of the stalks (see figures) were all feeding, not restless.

120 hours

- On the bottom segments, larvae that fed on root primordia were mostly on developing roots. N11 had 57% of the emerged larvae and N21, 53%.

The resistant varieties appeared to deter larvae more than others. In four time intervals out of six, they were the only varieties with high numbers trapped in glue. These are larvae that would probably fall off the plant under normal field conditions to feed on the trash and thus get exposed to predation by ants and spiders. N11 and N17 are rated among the very hairy varieties, which would explain the low numbers of larvae recovered from them.

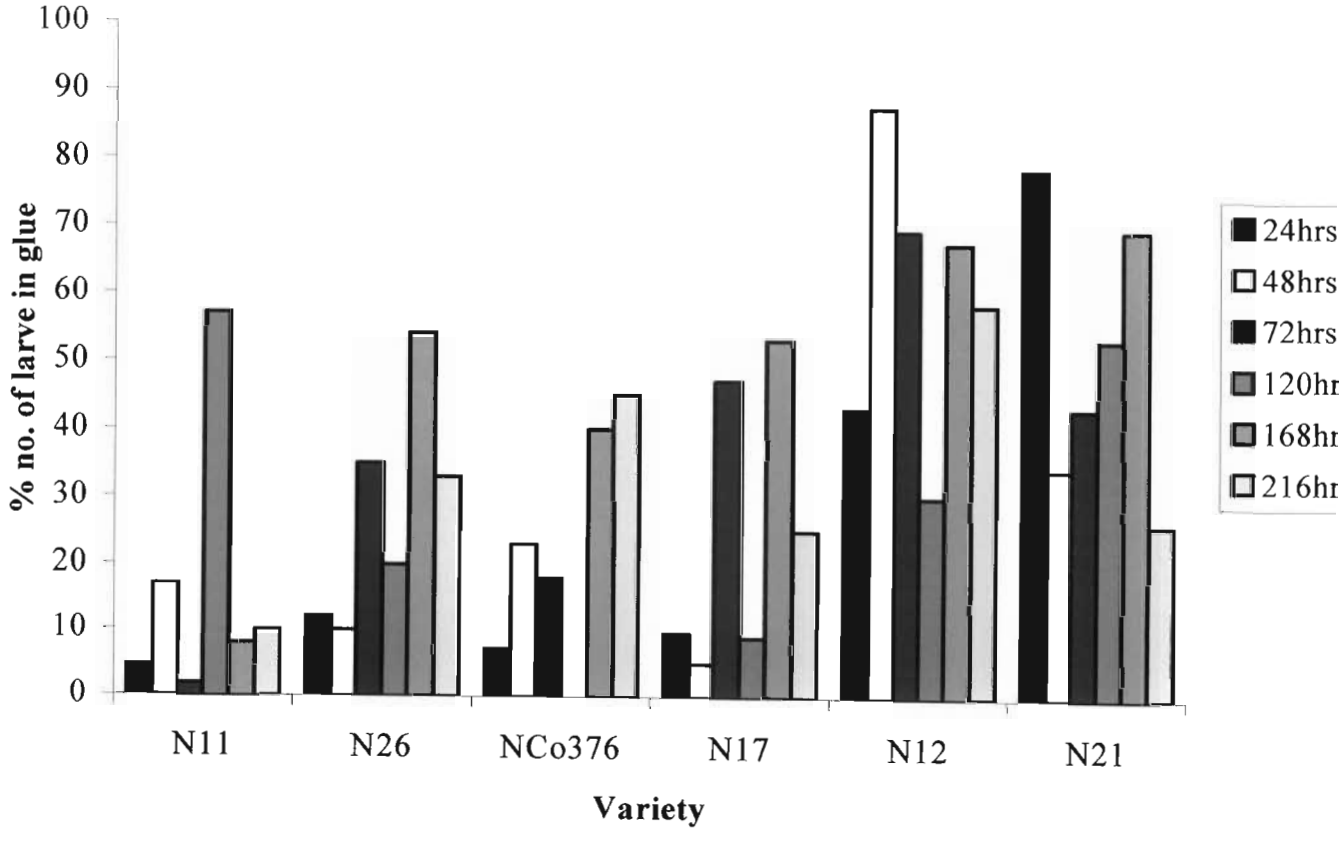

Figure 37: Percentage of larvae that fell of the plants and became trapped in glue.

Abbreviations on graphs:

B (prefix) = Bottom

M (prefix) = Middle

LB = Leaf Blade

LS = Leaf Sheath

Abxl = Abaxial surface

Adxl = Adaxial surface

Bd = Bud

RP = Root Primordia

LSc = Leaf Scar

Int = Internode

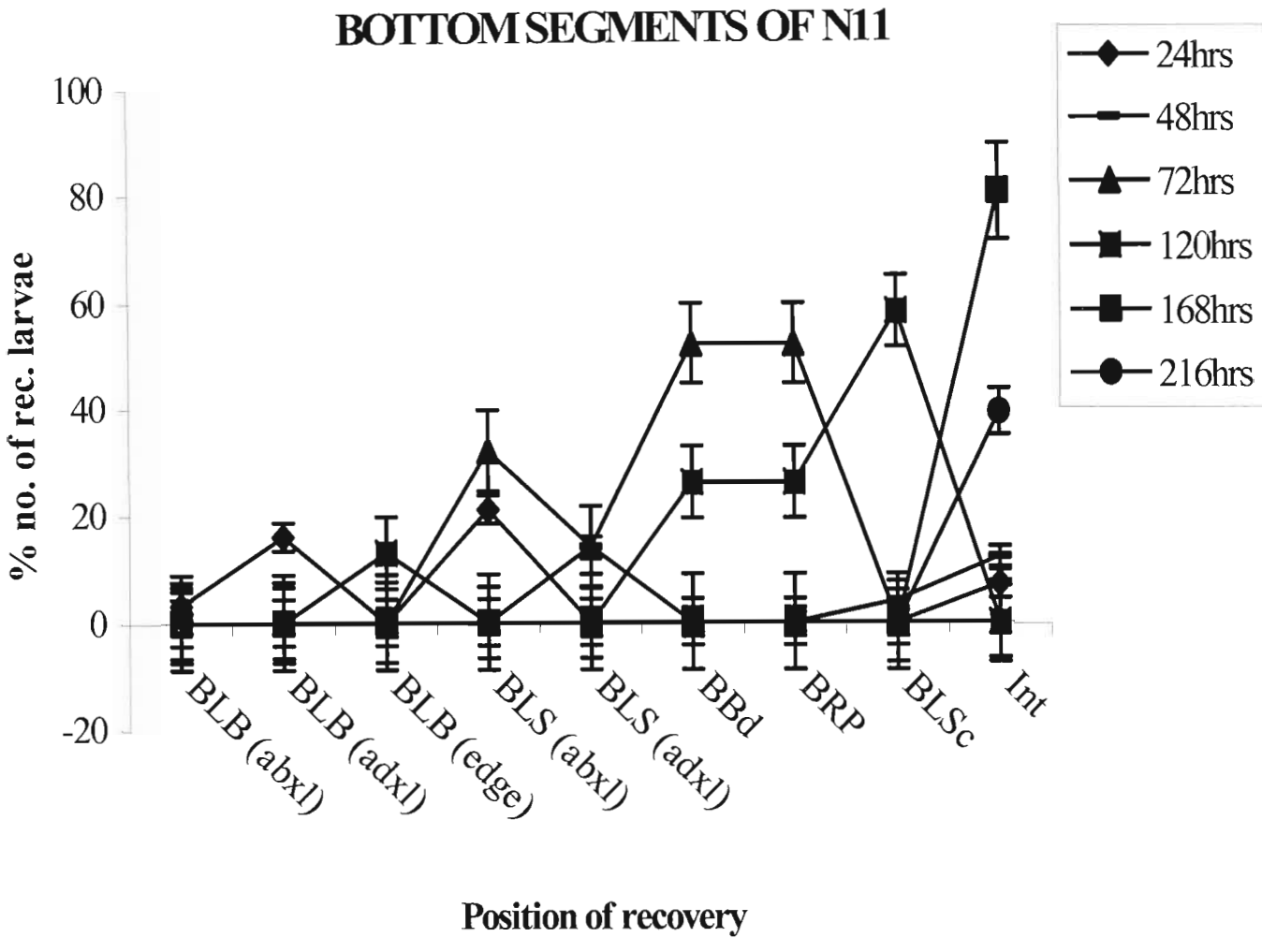

Figure 38: Positions of recovery on the bottom segments and percent number of larvae (bars = Error Bars) recovered from N11 after eggs were allowed to hatch and larvae to disperse at six different time intervals.

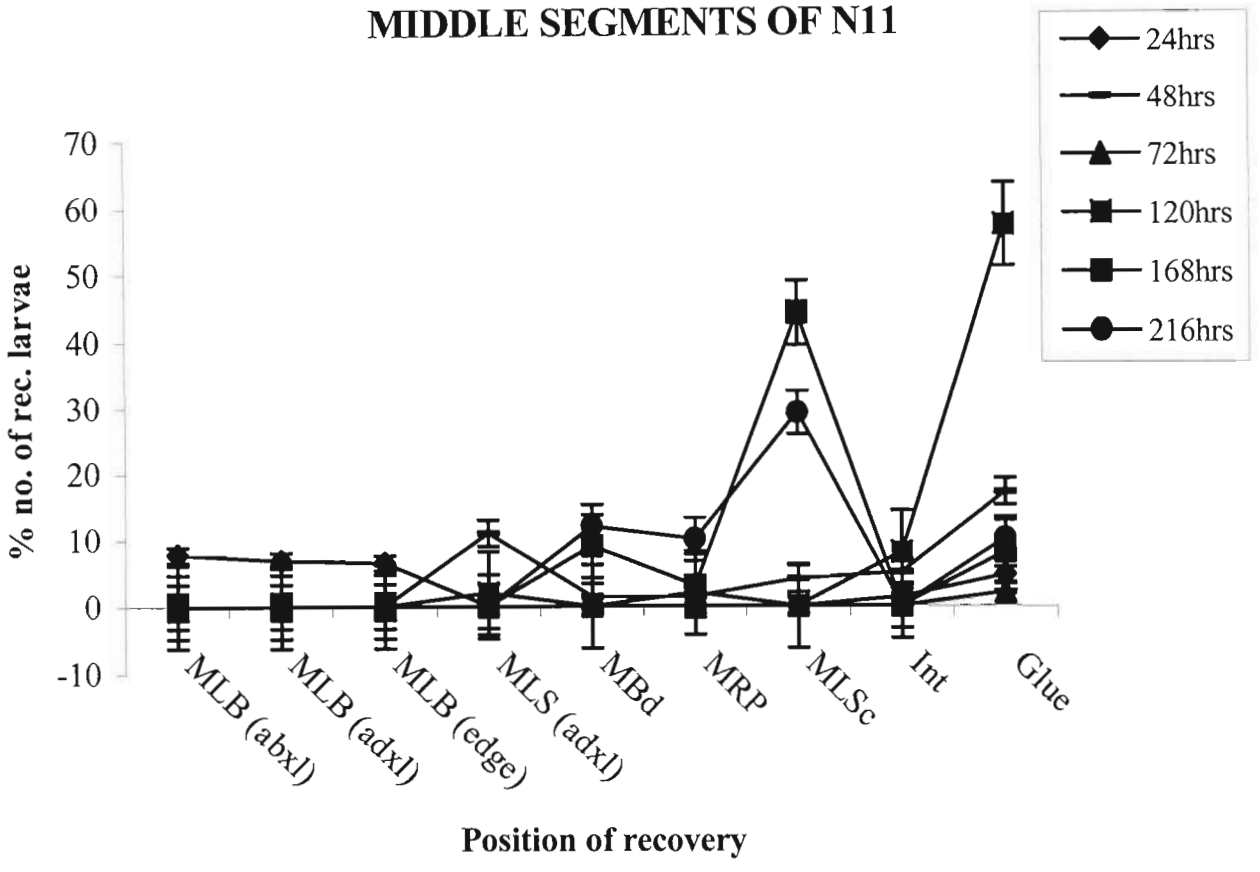

Figure 39: Positions of recovery on the middle segments and percent number of larvae (bars = Error Bars) recovered from N11 after eggs were allowed to hatch and larvae to disperse at six different time intervals.

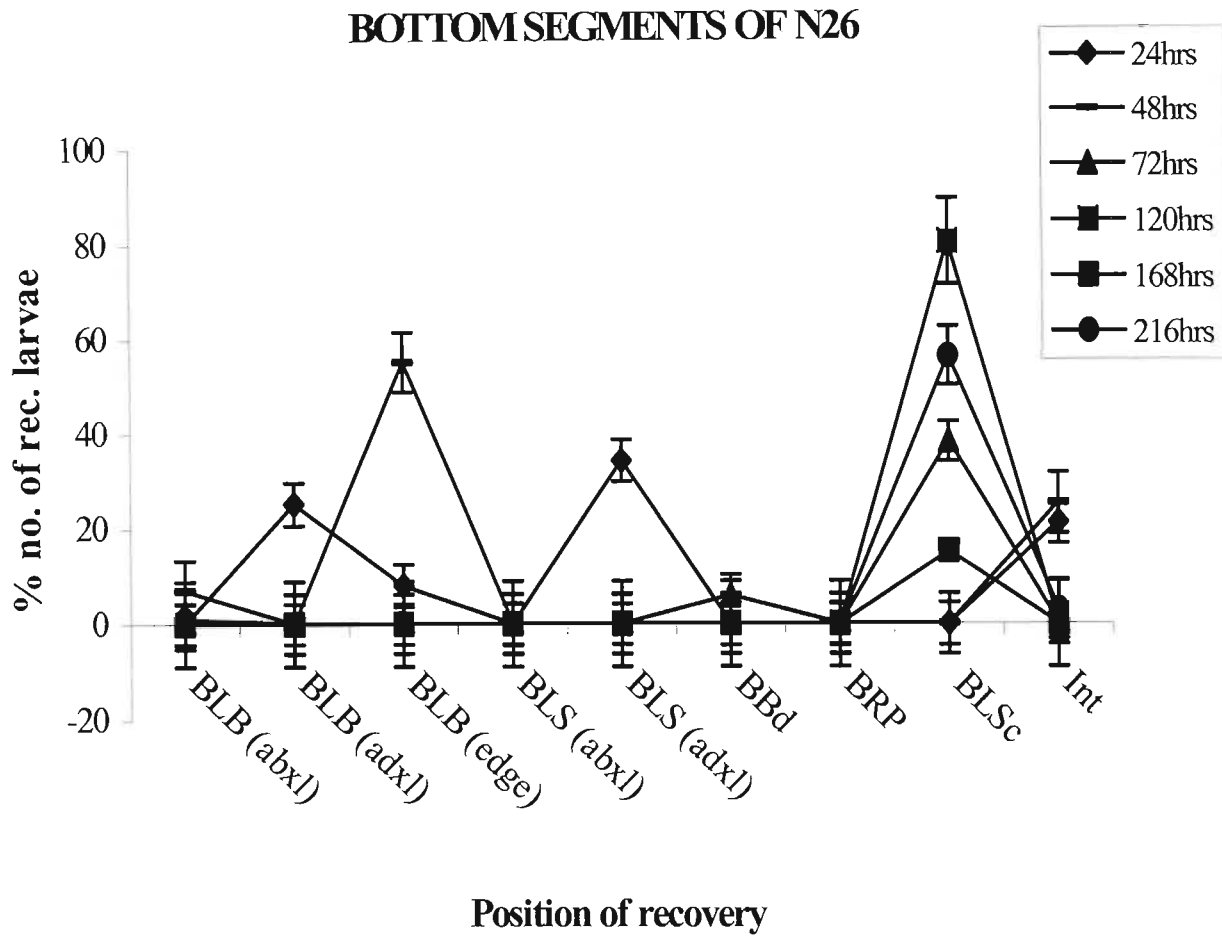

Figure 40: Positions of recovery on the bottom segments and percent number of larvae (bars = Error Bars) recovered from N26 after eggs were allowed to hatch and larvae to disperse at six different time intervals.

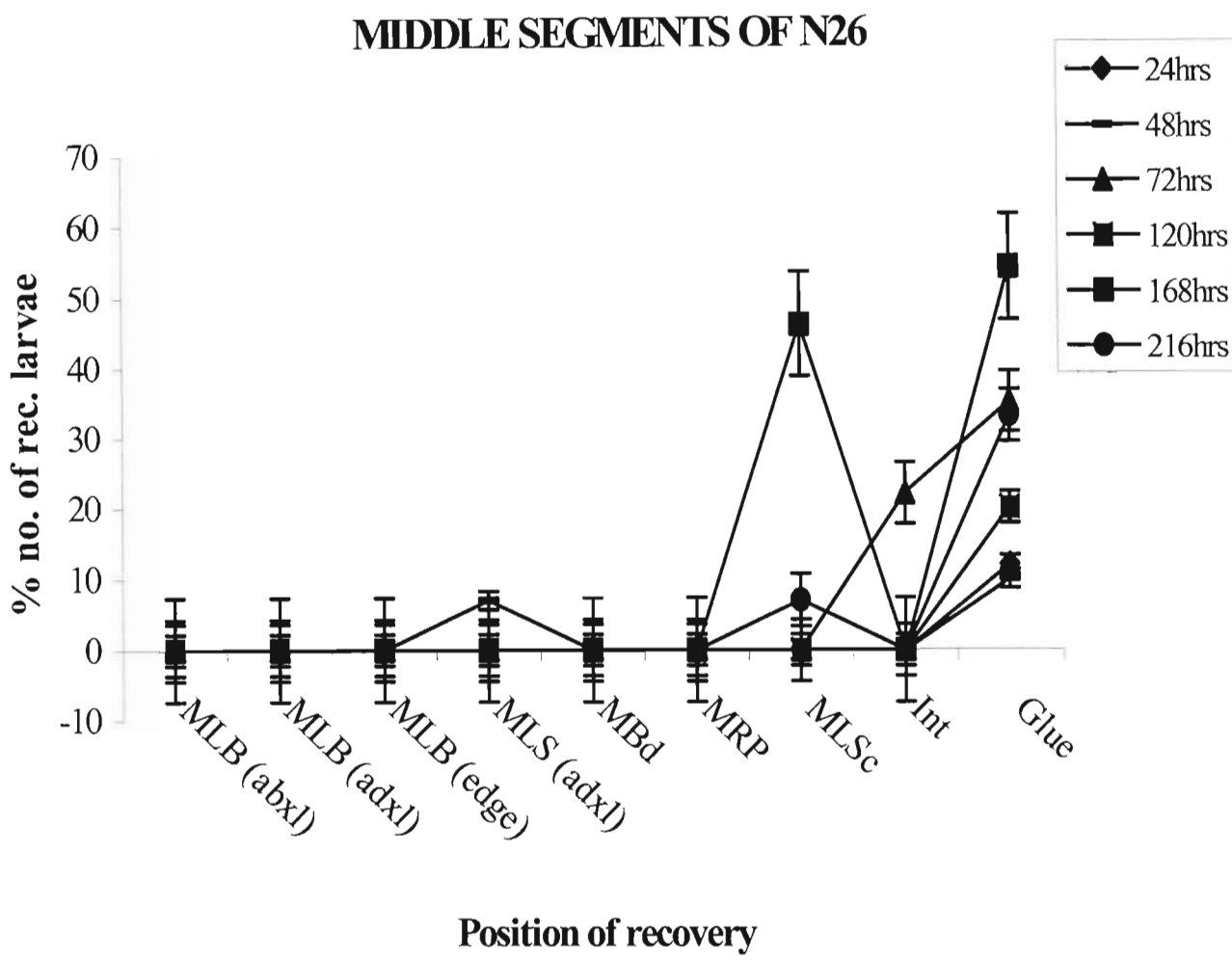

Figure 41: Positions of recovery on the middle segments and percent number of larvae (bars = Error Bars) recovered from N26 after eggs were allowed to hatch and larvae to disperse at six different time intervals.

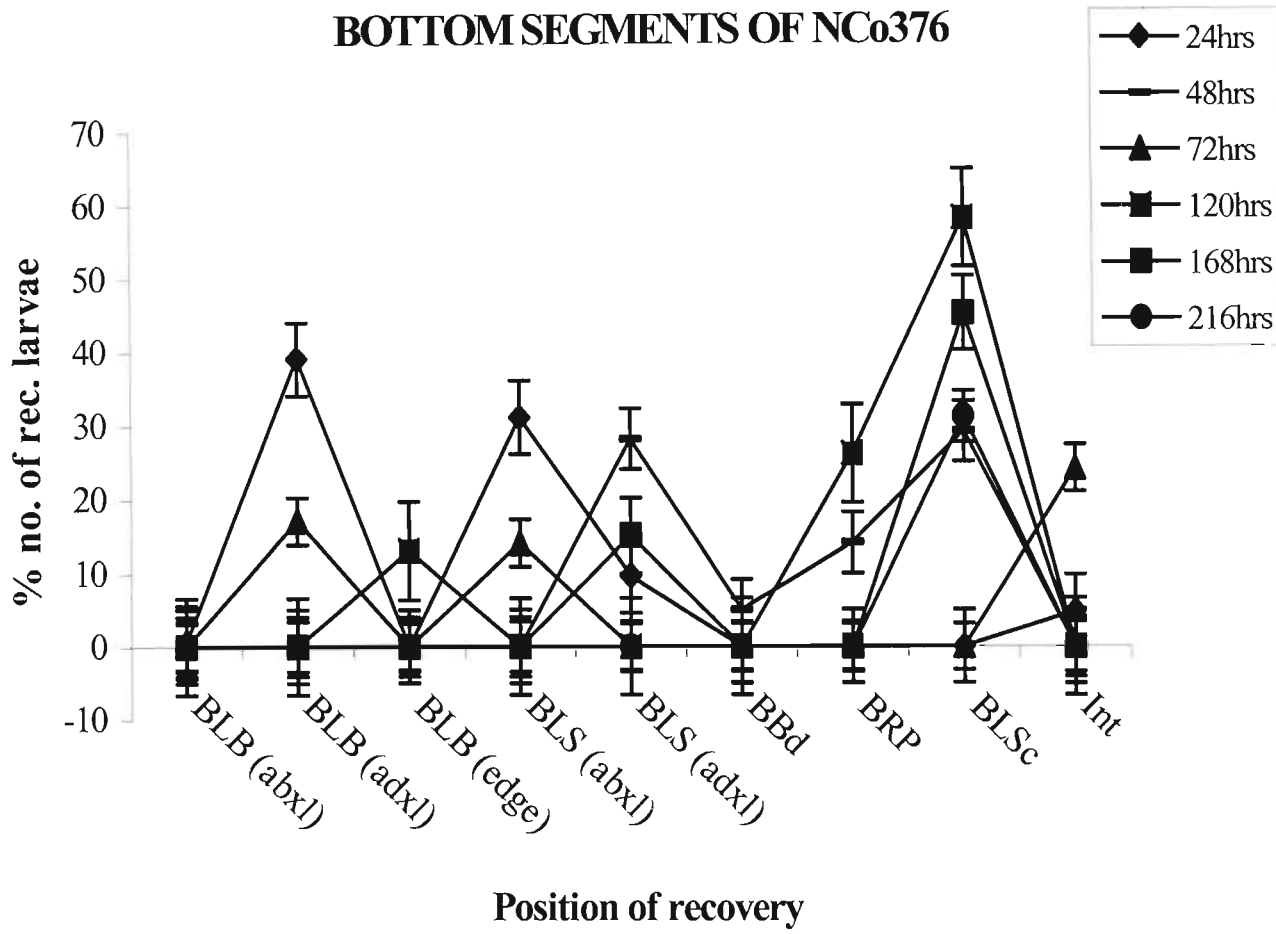

Figure 42: Positions of recovery on the bottom segments and percent number of larvae (bars = Error Bars) recovered from NCo376 after eggs were allowed to hatch and larvae to disperse at six different time intervals.

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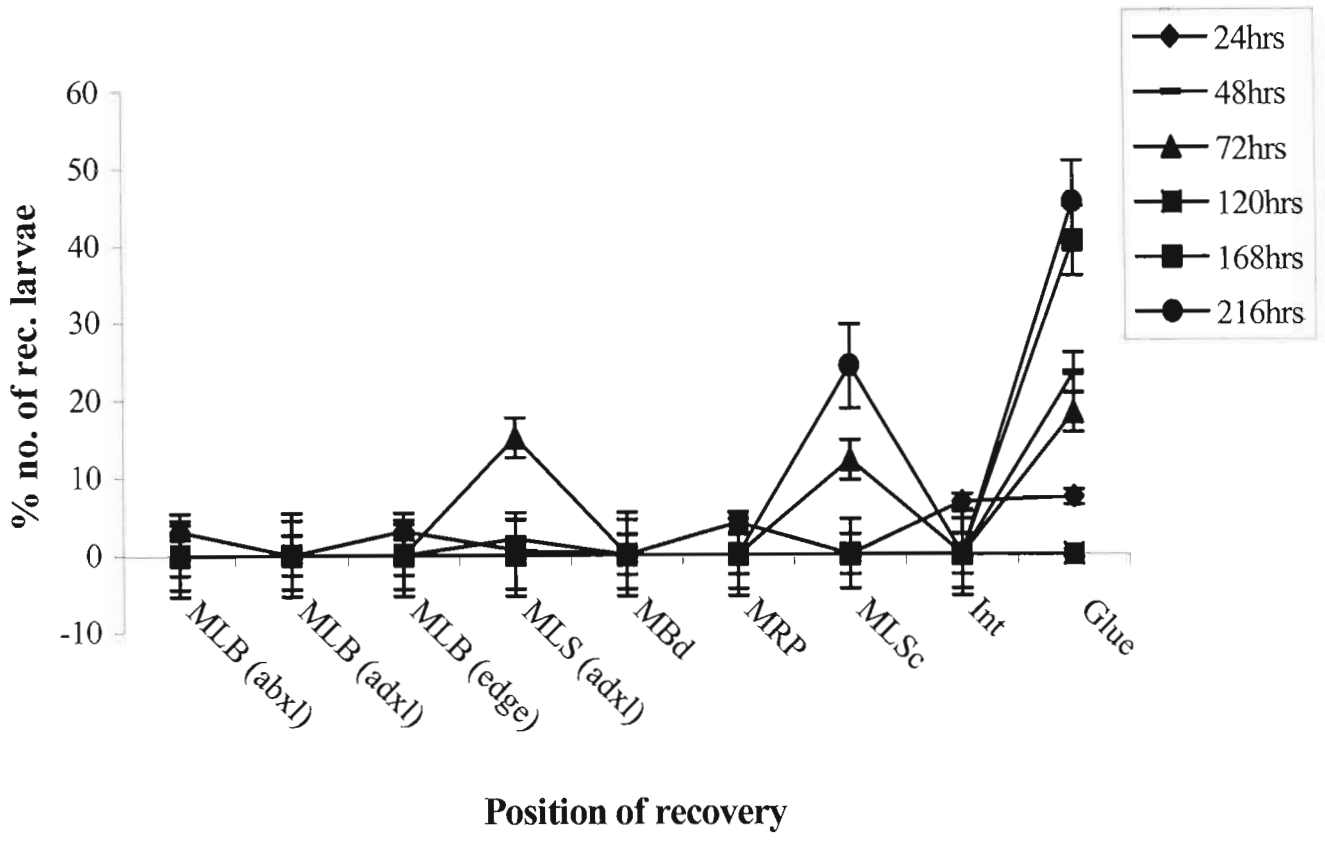

Figure 43: Positions of recovery on the middle segments and percent number of larvae (bars = Error Bars) recovered from NCo376 after eggs were allowed to hatch and larvae to disperse at six different time intervals.

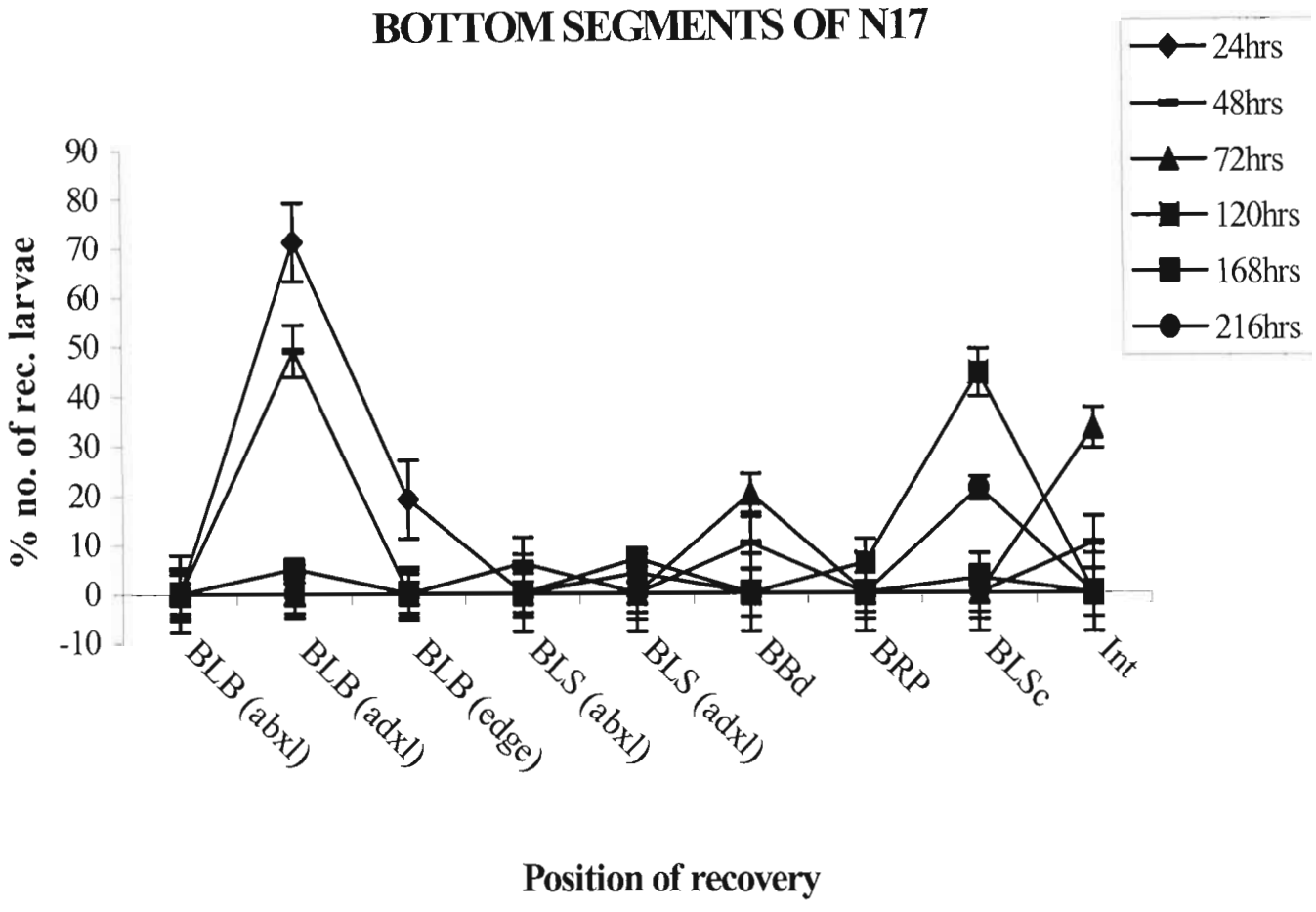

Figure 44: Positions of recovery on the bottom segments and percent number of larvae (bars = Error Bars) recovered from N17 after eggs were allowed to hatch and larvae to disperse at six different time intervals.

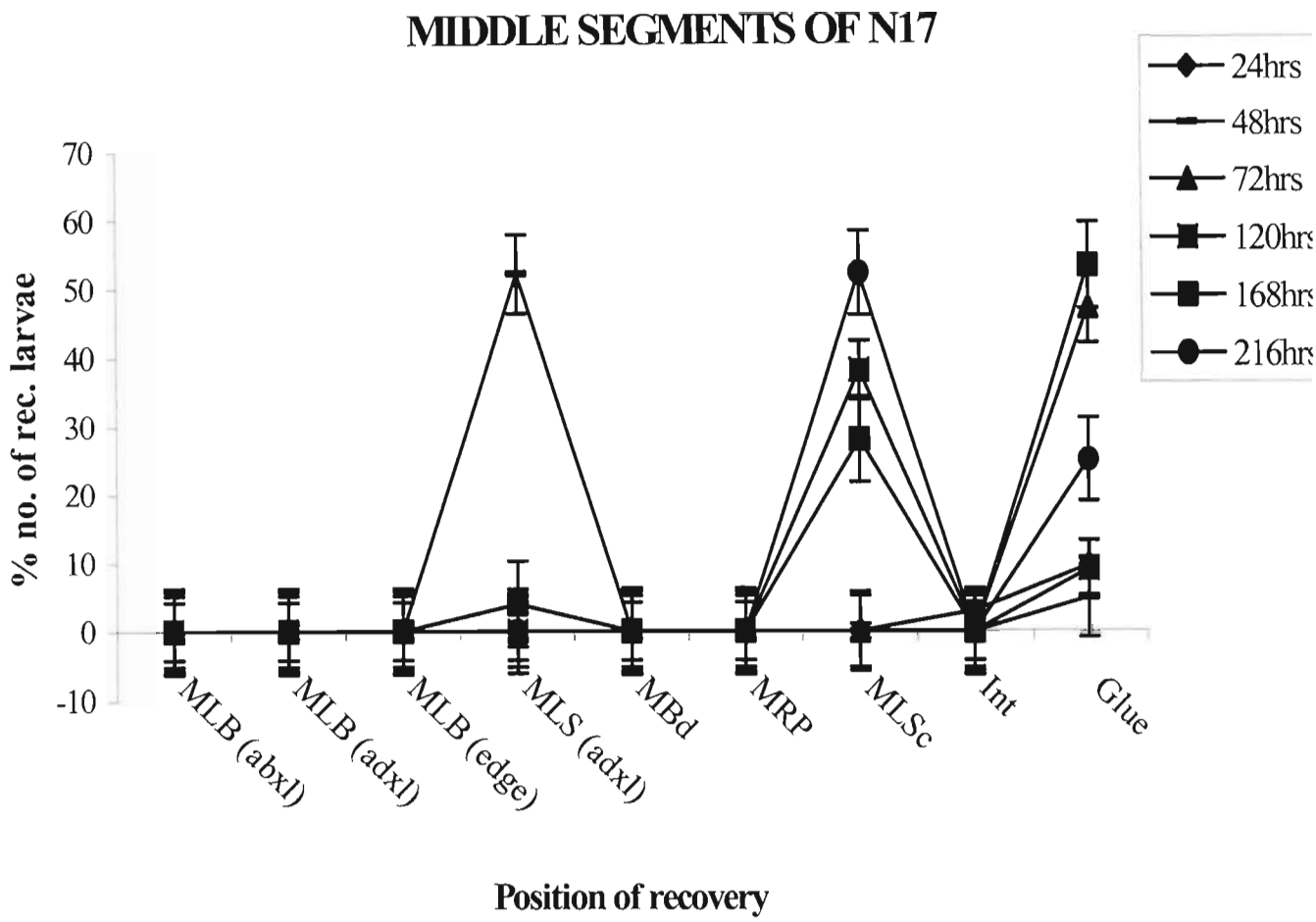

Figure 45: Positions of recovery on the middle segments and percent number of larvae (bars = Error Bars) recovered from N17 after eggs were allowed to hatch and larvae to disperse at six different time intervals.

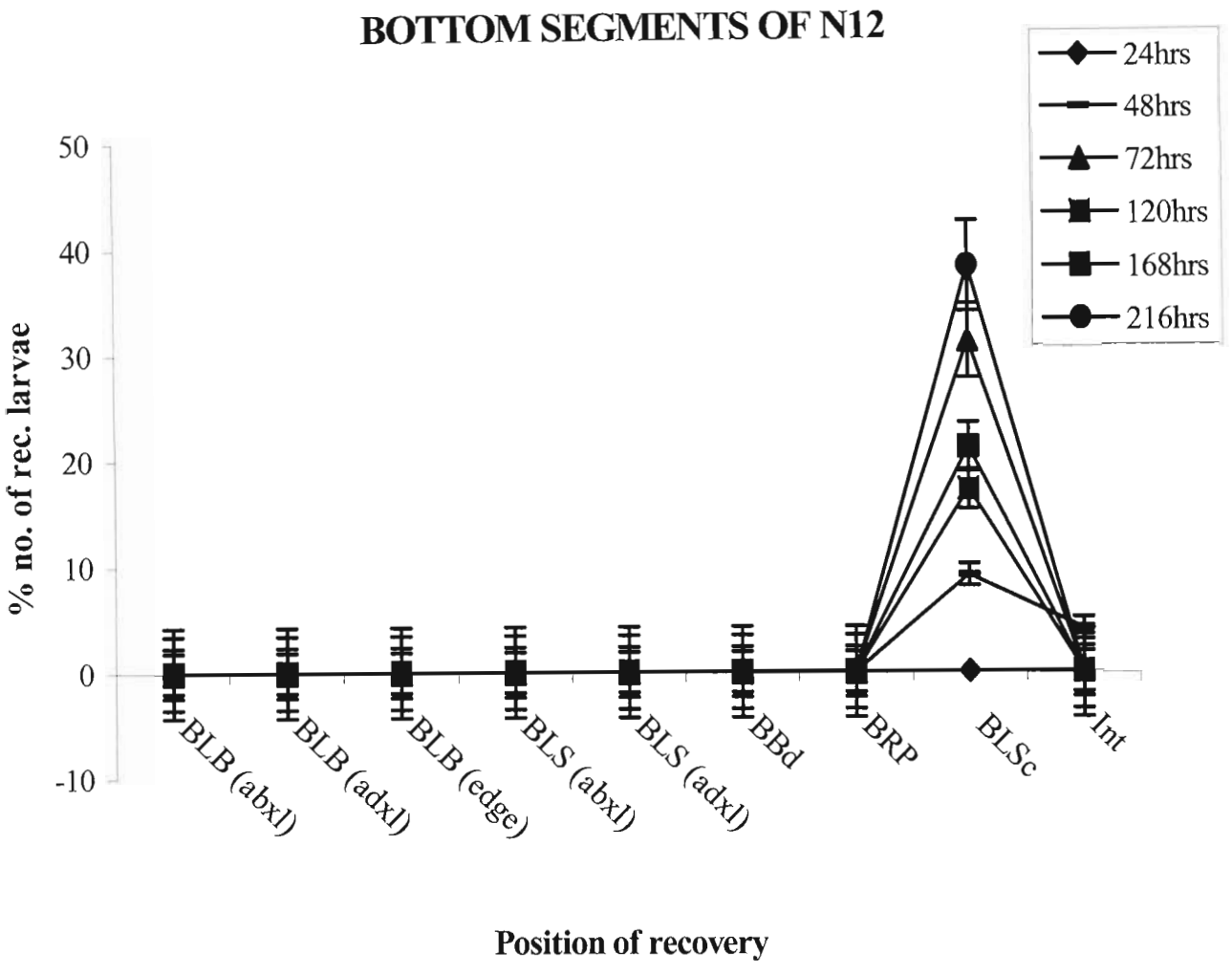

Figure 46: Positions of recovery on the bottom segments and percent number of larvae (bars = Error Bars) recovered from N12 after eggs were allowed to hatch and larvae to disperse at six different time intervals.

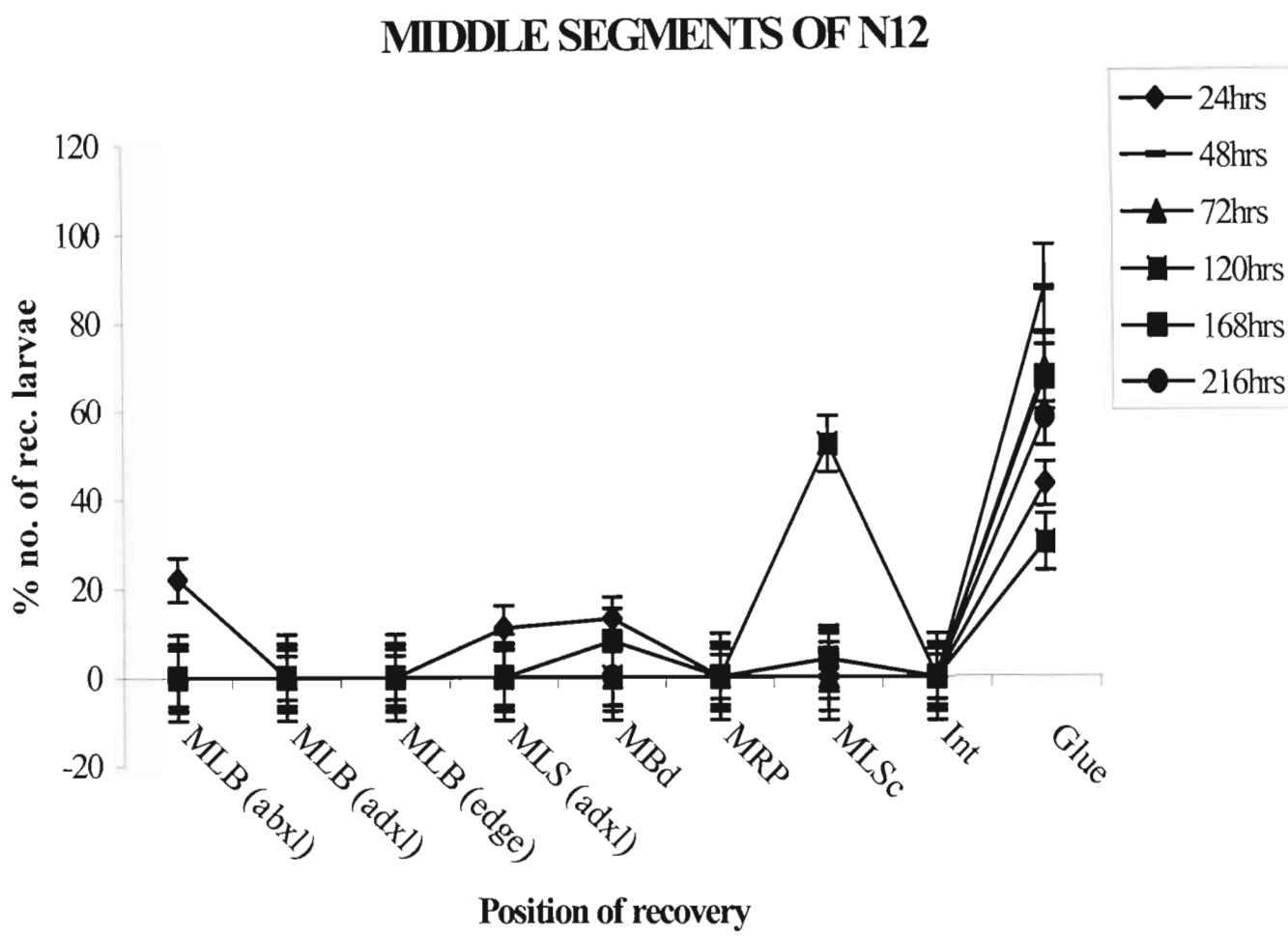

Figure 47: Positions of recovery on the middle segments and percent number of larvae (bars = Error Bars) recovered from N12 after eggs were allowed to hatch and larvae to disperse at six different time intervals.

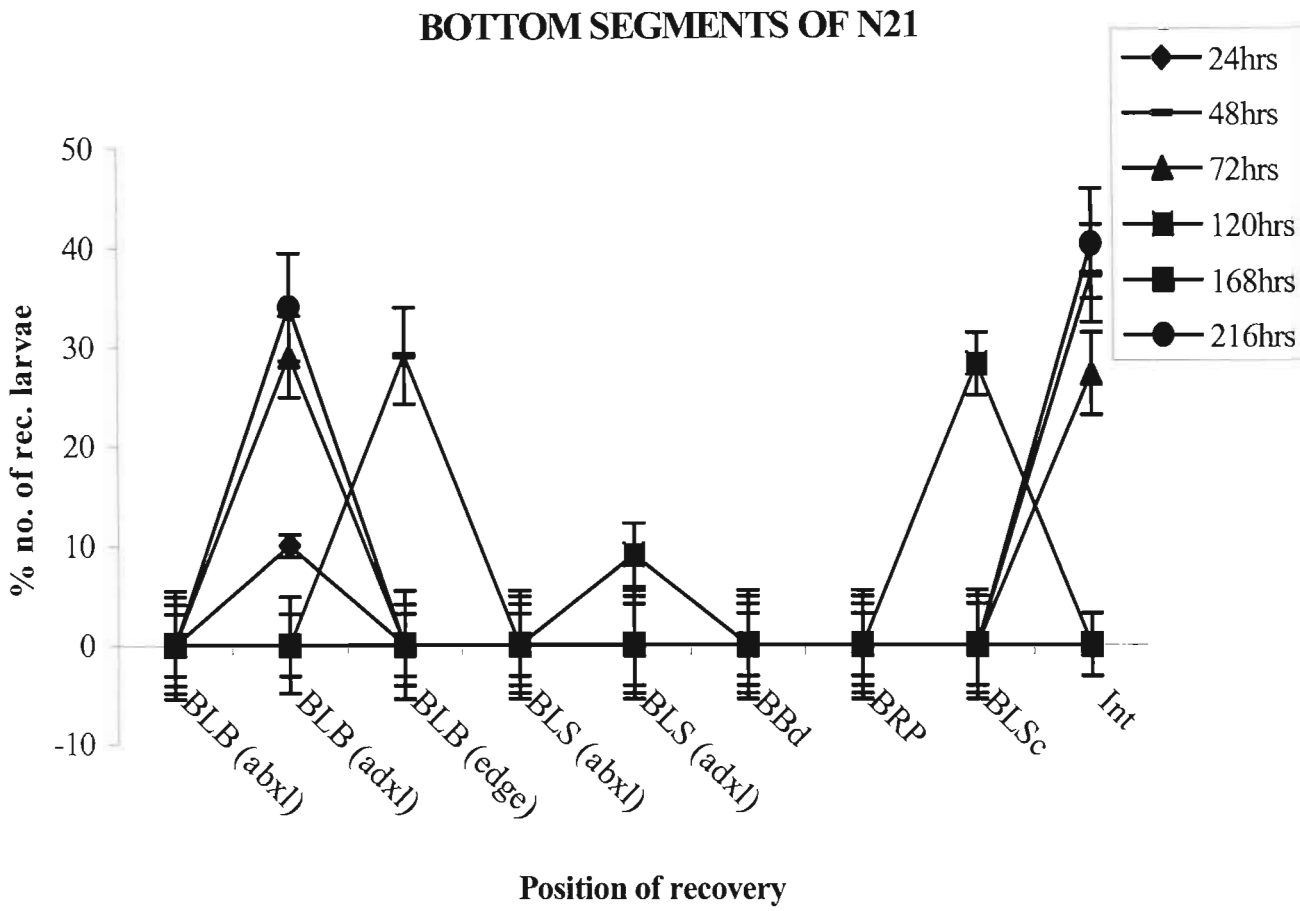

Figure 48: Positions of recovery on the bottom segments and percent number of larvae (bars = Error Bars) recovered from N21 after eggs were allowed to hatch and larvae to disperse at six different time intervals.

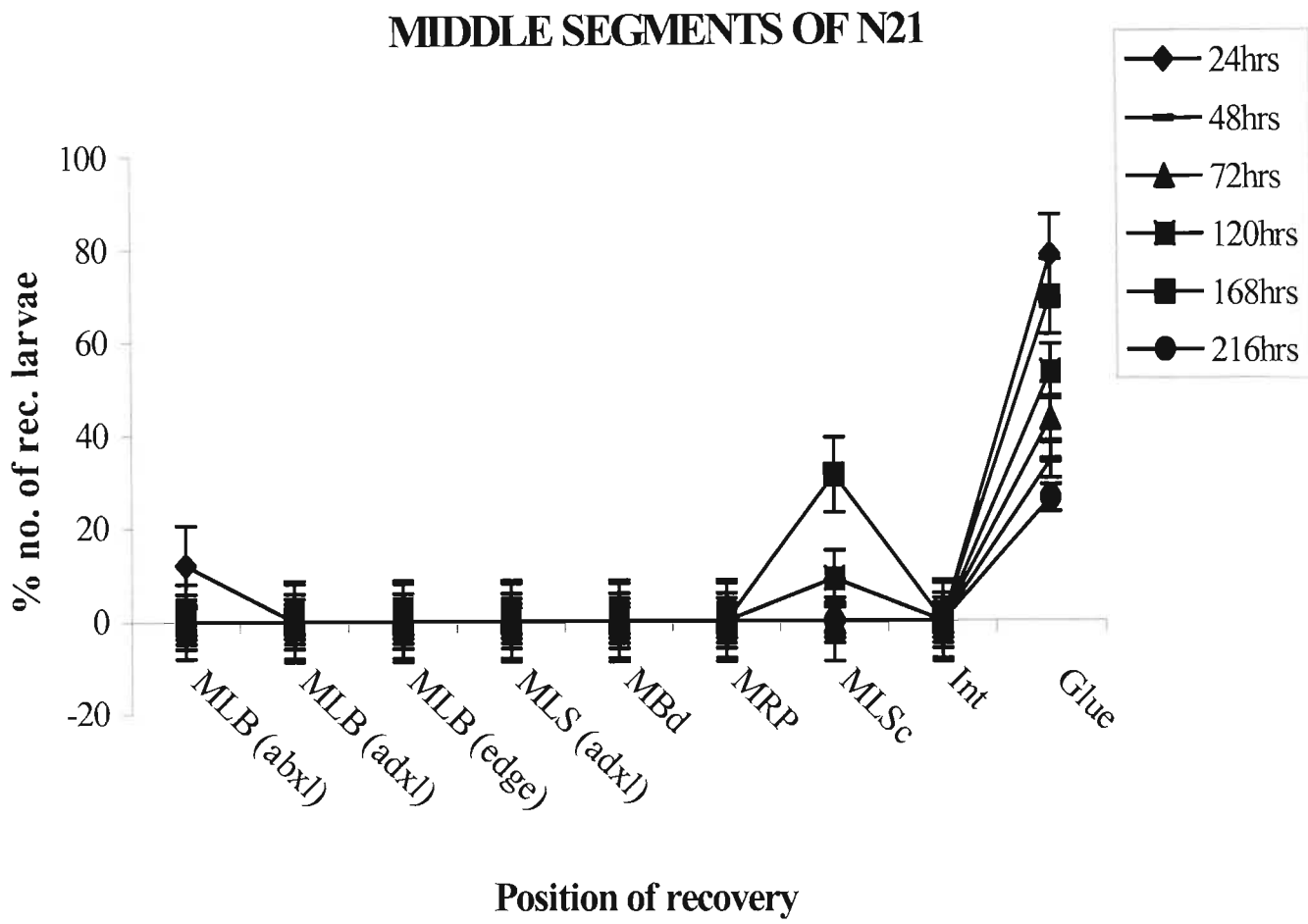

Figure 49: Positions of recovery on the middle segments and percent number of larvae (bars = Error Bars) recovered from N21 after eggs were allowed to hatch and larvae to disperse at six different time intervals.

Woodhead & Taneja (1987) assessed the importance of the initial phase of larval life on damage to sorghum, and whether it should be included in resistance screening methods. The cultivars were selected on the basis of their resistance to stem borer under natural and artificial infestation. IS 1151 was included because it had been used in earlier experiments for comparison with the resistant IS 2205 (Bernays *et al.*, 1983). In addition, three susceptible cultivars, ICSV 1, ICSV 2 and CSH1 were included. Since artificial infestation places live larvae directly at their feeding site, these differences may be due either to ovipositional non-preference, or to differences in initial larval establishment.

Low establishment is an effective resistance mechanism only in some cultivars, where it may be reinforced by other mechanisms operating in the later stages of larval life, or in some cases at oviposition. In other cultivars, resistance at the initial stage reduces the numbers of larvae becoming established but does not affect later susceptibility, suggesting a particularly favourable plant for larval development (Woodhead & Taneja, 1987).

Tightness of leaf sheath has also been cited as the cause of resistance of variety NCo310 to *D. saccharalis* (Kyle & Hensley, 1970). The first internode in all varieties is tightly enveloped by the leaf sheaths and the rind cannot be penetrated by larvae. In other internodes, in the succeeding vulnerable portion of a resistant variety like Co.J.46, the leaf sheaths up to the collar region fit tightly enough to prevent most of the early instar larvae from reaching their inner side wherein they feed on the sheath and tender internodes, preparatory to boring into the cane stalk. When these leaf sheaths were loosened artificially, more larvae established themselves. Thus the tightness of the leaf sheath is the first adverse barrier for internode borer entry in resistant varieties. In such varieties, sufficient water is held within the sheaths to drown successful larvae that manage to get inside (David & Joseph, 1982).

Leaf pubescence is a known morphological plant character that imparts resistance to insects in several crops (Sosa *et al.*, 1997). Sosa *et al.* (1997) tested 28 clones of *Saccharum robustum* for

pubescence. Crosses to incorporate pubescence into commercial cultivars began in 1984. Expression of pubescence varied greatly between crosses and among progenies of the same cross. Some plants exhibited pubescence on one leaf surface but not the other, and at different densities. Differences were also observed within the same leaf surface, being extremely pubescent close to the base (about 1/3 of the leaf length) to entirely absent on the remaining leaf area. The great advantage of pubescence as an insect resistant character in a breeding program is its visibility; no additional tests are needed to determine its effectiveness. A pubescent cultivar could be highly effective in reducing loses by sugarcane borers and possibly to other pests of sugarcane (Sosa *et al.*, 1997).

CONCLUSIONS

This study indicated that a large proportion of the variation in sugarcane varietal resistance to *E. saccharina* could be explained in terms of budscale hardness, rind hardness and surface waxes. There is a relationship between hard budscales, hard rind and unknown wax components. Although the results showed no clear indication that the resistance of certain varieties to *E. saccharina* is correlated to wax composition, trends were apparent. There was a clear indication that rind hardness and the budscale hardness are associated with varietal resistance and only affect first instar larvae.

Predation is adversely influenced by neonate mobility. The more time neonates spend wandering around on the stalk surface or exposed parts of the plant, the more vulnerable they are to predation and other adverse factors that may reduce their survival. Varieties with characteristics, which have not been identified, that promote larval mobility and slow larval establishment may be considered to display higher larval antixenosis (i.e. they reduce larval survival during the period of larval establishment/settling on the plant). This may be very important, since neonate survival is much lower than survival of later instars that have penetrated the stalk.

Significant differences were observed in the survival of larvae amongst varieties in most of the 'nodal' experiments. Second and third instar larvae were used subsequently to the mortality of all neonates fed on the rind, which in turn resulted in non-significant differences, suggesting that feeding on debris and/or leaves is critical to the survival and penetration of larvae into the sugarcane stalk.

In the screening experiments, first-instar larvae moved immediately from their points of release to the node and then into the leaf scar tissues, where they found refuge. Most of the larvae penetrated and fed on the bud and the rest through the leaf scar. Neonates that fed on the bud were heavier than those that fed below the leaf scar were.

In budscale hardness experiments, significant differences in larval masses among varieties in 'treatments' suggested differences among the physical or chemical properties of the bud and the budscale among varieties. When the budscale and/or the whole bud were removed, the larvae penetrated the stalks faster, suggesting that the budscale may impede penetration of the stalk to some extent. Trends for 'mass of larvae' feeding on different varieties were largely as expected based on known resistance of varieties, although NCo376 appeared more susceptible than intermediate in most cases.

In rind hardness experiments, second-instar larvae survived and fed as opposed to neonates, which proves that neonates need to feed on other sources of food before boring into the stalk. The presence of the rind appeared to be a crucial factor for the survival of early instars. When early instars were tested for feeding on the internode without the rind they did not show preference on any variety (there was no survival), but when the same experiment was repeated on the node with the removed rind, there was survival, although differences were non-significant. Third-instar larvae had no difficulty feeding on any of the varieties. Although third instars did not feed well on resistant varieties as they did on the susceptible ones, they still survived after seven days. First and second instars showed the complete opposite.

Larval infestations by *E. saccharina* on sugarcane could be reduced by making the stalk less favourable for first instar larvae and this could be done by breeding for varieties that possess the characteristics that were tested in this study. The main focus should be on early instars because leaf pubescence and leaf sheath tightness act specifically on them and thus increasing the success of other pest control methods such as chemical control. The rind is still effective in controlling survival of second instar larvae, so the larvae that get by the trichomes and past the leaf sheaths would still not be able to get into the node, which is their preferred point of feeding on the stalk. The aim is to produce varieties that possess most, if not all of the unfavourable characteristics, but still produce enough sucrose for the sugar industry. Incorporation of the characteristics tested in

these experiments aims to reduce the number of larvae that penetrate the stalk and to expose them for longer on the surface where their numbers may be controlled by predators and insecticides.

The results showed that larvae tend to be restless on hairy varieties, which are also susceptible (N11) and intermediate (N17), suggesting that larvae may have difficulty establishing on a plant due to physical characteristics, but once they escape the trichomes on leaf sheaths, they penetrate easily. The resistant varieties used in these experiments have high fibre and less sugar, but newer varieties, such as N29 and N33 incorporate both high resistance and high sucrose yield, which are the two key elements for optimised sugar production. Chemical characteristics of the plants need to be taken into consideration as high sucrose is seldom found in fibrous varieties. N8, N20 and N21 are considered highly resistant to *E. saccharina*, but are no longer used for sugar production, because of the insignificant amount of sucrose they yield. Leaf sheath tightness is another characteristic that would go well with leaf sheath hairiness, because though not tested in this work—would make it difficult for the larvae to get to the smooth adaxial surface of the leaf. The hardness of trichomes is another feature that needs to be investigated, because a variety may have dense trichomes that are not hard enough to repel even the most sensitive larvae, neonates. At present, breeding for the desired characteristics is the most important defence along with cultural control, i.e. field hygiene. Cultural control stresses cutting after 12 months in areas where fields are highly stressed to reduce the risk infestations above threshold from building up when the sugar mills are closed between December and April. Cutting early has its disadvantages though; growers are losing quite a lot, because there is significant accumulation of sucrose from 12 months onwards of the growing cycle. Breeding for plant resistance in sugarcane is the long-term, cost-beneficial solution.

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APPENDIX 1: Latin Square design for planting sugarcane plants used in all experiments

	Col 1		Col 2		Col 3		Col 4		Col 5		Col 6		Col 7		Col 8		Col 9	
	var	potno	var	potno	var	potno	var	potno	var	potno	var	potno	var	potno	var	potno	var	potno
Row1	N21	**12**	NCo376	**24**	N26	**36**	N17	**48**	N12	**60**	N11	**72**	N17	**84**	N12	**96**	N11	**108**
Row2	N12	**11**	N26	**23**	N17	**35**	N21	**47**	N11	**59**	NCo376	**71**	N11	**83**	NCo376	**95**	N12	**107**
Row3	N17	**10**	N21	**22**	N11	**34**	N26	**46**	NCo376	**58**	N12	**70**	NCo376	**82**	N11	**94**	N21	**106**
Row4	N26	**9**	N11	**21**	NCo376	**33**	N12	**45**	N17	**57**	N21	**69**	N12	**81**	N26	**93**	N17	**105**
Row5	NCo376	**8**	N17	**20**	N12	**32**	N11	**44**	N21	**56**	N26	**68**	N26	**80**	N21	**92**	NCo376	**104**
Row6	N11	**7**	N12	**19**	N21	**31**	NCo376	**43**	N26	**55**	N17	**67**	N21	**79**	N17	**91**	N26	**103**
Row7	N26	**6**	N21	**18**	N11	**30**	NCo376	**42**	N17	**54**	N12	**66**	N21	**78**	N26	**90**	NCo376	**102**
Row8	N17	**5**	N26	**17**	N12	**29**	N21	**41**	N11	**53**	NCo376	**65**	N26	**77**	N21	**89**	N17	**101**
Row9	NCo376	**4**	N17	**16**	N21	**28**	N12	**40**	N26	**52**	N11	**64**	N12	**76**	N17	**88**	N26	**100**
Row10	N12	**3**	N11	**15**	NCo376	**27**	N26	**39**	N21	**51**	N17	**63**	N11	**75**	NCo376	**87**	N21	**99**
Row11	N21	**2**	NCo376	**14**	N17	**26**	N11	**38**	N12	**50**	N26	**62**	N17	**74**	N12	**86**	N11	**98**
Row12	N11	**1**	N12	**13**	N26	**25**	NCo376	**37**	NCo376	**49**	N21	**61**	NCo376	**73**	N11	**85**	N12	**97**

LABORATORY

APPENDIX 2: Screening for resistance using stalk segments with 'One node'

Variety	Replicate	Position of boring	No of recovered larvae		Pooled mass of larvae
			Dead	Live	
NCo376	1		2		
	2			3	0.0959
	3			1	0.0009
	4	bud	1		0.0863
	5	bud	2	1	0.0691
	6		1		
Average					0.0631
N11	1	end-through wax	1		
	2		2		
	3		1		
	4				
	5	end-through wax	2		
	6	bud, leaf scar		1	0.0952
Average					0.0952
N12	1		2		
	2	end-through wax		2	0.0496
	3				
	4		3		
	5		1		
	6		1		
Average					0.0496
N17	1	end-through wax	1	2	0.0656
	2	leaf scar	1	1	0.0021
	3	end-through wax	1	1	0.0414
	4		3		
	5	leaf scar	1	1	0.0292
	6	leaf scar	1		
Average					0.0346
N21	1	bud		1	0.0304
	2		2		
	3	bud		1	0.0091
	4		2		
	5	leaf scar		1	0.0079
	6	end-through wax		1	0.1592
Average					0.0517
N26	1	end-through wax		1	0.0011
	2	leaf scar		3	0.1858
	3				
	4				
	5	leaf scar	1	1	0.0982
	6	leaf scar		1	0.1263
Average					0.1029

APPENDIX 3: Screening for resistance using stalk segments with 'Two nodes'

Variety	Replicate	Position of boring	No of recovered larvae		Pooled mass of larvae
			Dead	Live	
NCo376	1	bud	2	3	0.0027
	2	bud	4	1	0.0105
	3	bud	1	1	0.0111
	4	end-through wax	4	1	0.0012
	5	bud	3	2	0.0169
	6		5	0	
Average					**0.0085**
N11	1		5	0	
	2		5	0	
	3	end-through wax	1	1	0.0009
	4		5	0	
	5	primordia	4	1	0.0044
	6	primordia	1	2	0.0056
Average					**0.0036**
N12	1		5	0	
	2	end-through wax	4	1	0.0004
	3	primordia	2		
	4		5		
	5	bud	1	2	0.0029
	6	end-through wax	1	1	0.0007
Average					**0.0013**
N16	1	end-through wax	4	1	0.0004
	2		5		
	3		5		
	4		5		
	5	leaf scar	1	2	0.0009
	6		5		
Average					**0.0007**
N17	1	primordia	4	1	0.0010
	2	end-through wax	4	1	0.0003
	3	leaf scar	4	1	0.0015
	4	end-through wax	1	4	0.0076
	5	primordia	2	3	0.0036
	6	primordia	1	3	0.0031
Average					**0.0029**
N21	1		5		
	2	bud	4	1	0.0012
	3	primordia	4	1	0.0024
	4		5		
	5	bud	4	1	0.0008
	6	primordia	2		
Average					**0.0015**

APPENDIX 4: Nodes and Internodes as points of larval stalk penetration

Variety	Replicate	Internode only	Internode with node
NCo376	1	--	0.0105
NCo376	2	--	0.0019
NCo376	3	--	0.0108
NCo376	4	--	0.0022
NCo376	5	--	--
NCo376	6	--	0.003
N11	1	--	0.0059
N11	2	--	--
N11	3	0.0004	0.0015
N11	4	--	--
N11	5	--	--
N11	6	0.0003	--
N12	1	--	--
N12	2	--	0.0009
N12	3	--	0.0009
N12	4	--	0.0026
N12	5	0.0004	--
N12	6	--	0.0004
N16	1	--	--
N16	2	--	0.0006
N16	3	--	--
N16	4	--	0.0018
N16	5	--	0.0013
N16	6	--	--
N17	1	--	0.0054
N17	2	--	--
N17	3	--	0.0012
N17	4	--	0.0008
N17	5	--	0.0064
N17	6	--	0.0059
N21	1	--	0.0059
N21	2	--	--
N21	3	--	0.003
N21	4	--	--
N21	5	--	--
N21	6	--	0.0014

APPENDIX 5: Feeding on root primordia

Variety	Replicate	Length of boring (cm)
NCo376	1	
	2	0.5
	3	0.2
	4	
	5	0.1
	6	0.3
N11	1	
	2	0.4
	3	
	4	0.3
	5	0.15
	6	
N12	1	
	2	
	3	
	4	
	5	
	6	
N16	1	
	2	
	3	
	4	
	5	
	6	0.5
N17	1	
	2	
	3	0.5
	4	
	5	0.3
	6	0.4
N21	1	
	2	
	3	
	4	0.4
	5	
	6	0.5

APPENDIX 6: Budscale removed (as treatment) and intact (as control)

Variety	Replicate	Mass of larvae after feeding	
		Treatment	Control
NCo376	1	0.0012	
	2	0.0019	
	3	0.0097	0.0050
	4	0.0034	0.0040
	5	0.0079	0.0071
	6	0.0065	0.0052
Average		0.0051	0.0053
N11	1	0.0063	
	2	0.0020	
	3	0.0040	0.0021
	4	0.0042	0.0039
	5	0.0082	
	6	0.0035	0.0029
Average		0.0047	0.0030
N12	1	0.0048	
	2	0.0018	0.0003
	3	0.0022	
	4	0.0015	
	5	0.0015	0.0033
	6	0.0009	
Average		0.0021	0.0018
N16	1	0.0036	
	2	0.0070	0.0048
	3	0.0009	0.0010
	4	0.0030	
	5	0.0029	0.0015
	6	0.0015	0.0009
Average		0.0032	0.0021
N17	1	0.0043	0.0063
	2	0.0035	0.0028
	3	0.0040	0.0021
	4	0.0038	0.0027
	5	0.0045	
	6	0.0027	0.0025
Average		0.0038	0.0033
N21	1	0.0022	0.0014
	2	0.0021	
	3		
	4	0.0013	
	5	0.0005	0.001
	6	0.0016	0.0012
Average		0.0015	0.0012

APPENDIX 7: Bud completely removed (treatment) and intact (control)

Variety	Replicate	Mass of larvae after feeding	
		Treatment	Control
NCo376	1	0.0205	0.0019
	2	0.0219	0.0181
	3	0.0224	0.017
	4	0.0217	0.012
	5	0.0162	0.0012
	6	0.0152	0.0137
Average		**0.01965**	**0.01065**
N11	1	0.0128	0.0022
	2	0.0077	0.0041
	3	0.0171	
	4		0.0016
	5	0.0124	
	6	0.0085	0.0105
Average		**0.0117**	**0.0046**
N12	1	0.0114	
	2	0.0029	0.0009
	3	0.0036	
	4	0.0007	
	5	0.0075	
	6	0.0003	0.0009
Average		**0.0044**	**0.0009**
N16	1	0.0092	
	2	0.0091	0.0041
	3	0.0051	0.0009
	4	0.0102	0.0057
	5	0.0088	0.0026
	6	0.0157	0.0011
Average		**0.00968**	**0.00288**
N17	1	0.0067	0.0051
	2	0.0062	0.0065
	3	0.0068	0.0027
	4		0.0013
	5	0.0083	
	6	0.0065	
Average		**0.00690**	**0.0039**
N21	1	0.0027	0.0015
	2	0.0117	0.0038
	3	0.0039	
	4	0.0051	0.0029
	5	0.0043	0.0012
	6		
Average		**0.00554**	**0.00235**

APPENDIX 8: Bud (treatment) and budscale (control) removed

Variety	Replicate	Mass of larvae after feeding	
		Treatment	Control
NCo376	1		
	2	0.0100	
	3	0.0050	
	4		
	5		
	6	0.0200	0.0200
Average		**0.0120**	**0.0200**
N11	1		
	2	0.0300	0.0200
	3		
	4	0.0200	0.0100
	5	0.0100	0.0100
	6	0.0200	
Average		**0.0200**	**0.0130**
N12	1		
	2	0.0030	0.0100
	3	0.0030	
	4		0.0040
	5		0.0020
	6		0.0100
Average		**0.0030**	**0.0060**
N16	1		0.0100
	2	0.1000	
	3	0.0100	0.0100
	4		0.0100
	5	0.0100	
	6	0.0050	0.0200
Average		**0.0310**	**0.0130**
N17	1	0.0050	
	2	0.0020	0.0040
	3		
	4	0.0100	
	5	0.0050	0.0100
	6		
Average		**0.0050**	**0.0070**
N21	1	0.0020	
	2		
	3		
	4		0.0100
	5	0.0100	
	6	0.0020	0.0030
Average		**0.0050**	**0.0060**

APPENDIX 9: Rind Hardness- using 2nd instar larvae

Variety	Rep	Mass of larvae before	Mass of larvae after feeding	Mass gained/lost
NCo376	1	0.0077	0.0144	0.0067
	2	0.0054	0.0105	0.0051
	3	0.0135	0.0268	0.0133
	4	0.0046	0.0108	0.0062
	5	0.006	0.0102	0.0042
	6	0.0047	0.0096	0.0049
Average		**0.00698**	**0.01372**	**0.00673**
N11	1	0.0063	0.0128	0.0065
	2	0.014	0.0219	0.0079
	3	0.0068	0.0174	0.0106
	4	0.0063	0.0121	0.0058
	5	0.0045	0.0104	0.0059
	6	0.0051	0.0092	0.0041
Average		**0.00717**	**0.01397**	**0.00680**
N12	1	0.0141	0.0154	0.0013
	2	0.0081	0.0159	0.0078
	3	0.0132	0.0106	-0.0026
	4	0.014	0.0111	-0.0029
	5	0.005	0.008	0.003
	6	0.0095	0.0181	0.0086
Average		**0.01065**	**0.01318**	**0.00253**
N16	1	0.0117	0.0189	0.0072
	2	0.0154	0.0233	0.0079
	3	0.0048	0.0074	0.0026
	4	0.0106	0.0132	0.0026
	5	0.0164	0.0176	0.0012
	6	0.0195	0.0304	0.0109
Average		**0.01307**	**0.01847**	**0.00540**
N17	1	0.0074	0.0158	0.0084
	2	0.0046	0.0116	0.007
	3	0.0045	0.019	0.0145
	4	0.0061	0.0179	0.0118
	5	0.0099	0.0214	0.0115
	6	0.0076	0.0197	0.0121
Average		**0.00668**	**0.01757**	**0.01088**
N21	1	0.0208	0.0312	0.0104
	2	0.0077	0.0158	0.0081
	3	0.0136	0.0186	0.005
	4	0.0056	0.0138	0.0082
	5	0.0139	0.0228	0.0089
	6	0.0054	0.0128	0.0074
Average		**0.01117**	**0.01917**	**0.00800**

APPENDIX 10: Rind Hardness- using 3rd instar larvae

		Rind removed			Rind intact		
Variety	**Replicate**	**Mass of larvae before**	**Mass of larvae after feeding**	**Mass gained/lost**	**Mass of larvae before**	**Mass of larvae after feeding**	**Mass gained/lost**
NCo376	1	0.085	0.0695	-0.0155	0.059		
	2	0.0448	0.0454	0.0006	0.0612	0.0567	-0.059
	3	0.0914	0.06	-0.0314	0.0504	0.0691	-0.0045
	4	0.1442	0.1041	-0.0401	0.1047	0.1364	0.0187
	5	0.0776	0.1157	0.0381	0.077	0.1045	0.0317
	6	0.0759	0.1213	0.0454	0.0619	0.0496	0.0275
stdev				**0.035626**			**0.037318**
Average				**0.004675**			**0.00288**
N11	1	0.125	0.087	-0.038	0.0841	0.0452	-0.0123
	2	0.1222	0.095	-0.0272	0.0807	0.0464	-0.0389
	3	0.0695	0.1067	0.0372	0.0579	0.0309	-0.0343
	4	0.0678	0.1033	0.0355	0.085		
	5	0.0675	0.0774	0.0099	0.1202		
	6	0.0716	0.1114	0.0398	0.0516	0.0872	-0.1202
stdev				**0.034539**			**0.047297**
Average				**0.009533**			**-0.05143**
N12	1	0.0712	0.1007	0.0295	0.0809	0.0902	0.0356
	2	0.1466	0.102	-0.0446	0.0612	0.0759	0.0093
	3	0.0447	0.0586	0.0139	0.0753	0.0682	0.0147
	4	0.1374	0.0637	-0.0737	0.0838	0.0469	-0.0071
	5	0.0305	0.0378	0.0073	0.0525		
	6	0.0721	0.069	-0.0031	0.0468	0.0521	-0.0525
stdev				**0.039279**			**0.033078**
Average				**-0.01178**			**-1.4E-18**
N16	1	0.0768	0.0929	0.0161	0.0535	0.0701	0.0053
	2	0.0506	0.0734	0.0228	0.0846	0.1349	0.0166
	3	0.0632	0.0821	0.0189	0.066	0.0843	0.0503
	4	0.1064	0.1087	0.0023	0.0904	0.0419	0.0183
	5	0.1191	0.0927	-0.0264	0.1067	0.1381	-0.0485
	6	0.0474	0.0642	0.0168	0.0928	0.1012	0.0314
stdev				**0.018416**			**0.033493**
Average				**0.008417**			**0.012233**
N17	1	0.1199	0.1077	-0.0122	0.0571		
	2	0.1562	0.1008	-0.0554	0.0657		
	3	0.0518	0.0879	0.0361	0.0518	0.0655	-0.0657
	4	0.0825	0.0887	0.0062	0.0435	0.0908	0.0137
	5	0.0579	0.0593	0.0014	0.0472	0.0759	0.0473
	6	0.0605	0.0889	0.0284	0.0719	0.0803	0.0287
stdev				**0.032756**			**0.049737**
Average				**0.00075**			**0.006**
N21	1	0.0351	0.0622	0.0271	0.0348	0.0224	0.0084
	2	0.0843	0.0955	0.0112	0.0406		
	3	0.1148	0.103	-0.0118	0.044		
	4	0.1304	0.0824	-0.048	0.0361	0.0205	-0.044
	5	0.0481	0.0651	0.017	0.0391	0.0492	-0.0156
	6	0.0791	0.0939	0.0148	0.0297	0.0355	0.0101
stdev				**0.027544**			**0.025362**
Average				**0.001717**			**-0.01028**

APPENDIX 11: Diet incorporation

Variety	Replicate	Live	Dead	Collective mass of larvae	Average mass of each larva
NCo376	1	51	13	0.9993	0.0196
NCo376	2	57	7	1.261	0.0221
NCo376	3	59	5	0.9319	0.0158
NCo376	4	56	8	1.084	0.0194
NCo376	5	60	4	1.4421	0.0240
NCo376	6	45	19	0.6428	0.0143
N11	1	54	10	1.4096	0.0261
N11	2	52	12	1.567	0.0301
N11	3	56	8	1.6014	0.0286
N11	4	61	3	1.4741	0.0242
N11	5	62	2	1.3831	0.0223
N11	6	64	0	2.1561	0.0337
N12	1	64	0	1.2948	0.0202
N12	2	55	9	1.3913	0.0253
N12	3	52	12	1.1292	0.0217
N12	4	39	25	0.753	0.0193
N12	5	54	10	1.1694	0.0217
N12	6	64	0	2.0906	0.0327
N16	1	62	2	0.7158	0.0115
N16	2	61	3	0.5372	0.0088
N16	3	62	2	0.6077	0.0098
N16	4	59	5	0.7417	0.0126
N16	5	56	8	0.3565	0.0064
N16	6	55	9	0.6178	0.0112
N17	1	48	16	1.1717	0.0244
N17	2	55	9	1.6118	0.0293
N17	3	50	14	1.782	0.0356
N17	4	38	26	1.1477	0.0302
N17	5	55	9	1.4499	0.0264
N17	6	50	14	1.165	0.0233
N21	1	63	1	1.3683	0.0217
N21	2	53	11	1.3933	0.0263
N21	3	57	7	1.9089	0.0335
N21	4	58	6	1.3494	0.0233
N21	5	46	18	1.523	0.0331
N21	6	54	10	1.8255	0.0338

GLASSHOUSE

APPENDIX 12: Budscale removed (as treatment) and intact (as control)

Variety	Replicate	Mass of larvae after feeding	
		Treatment	Control
NCo376	1	0.0012	
	2	0.0019	
	3	0.0100	0.0100
	4	0.0034	
	5	0.0100	0.0100
	6	0.0100	0.0100
Average		**0.0061**	**0.0100**
N11	1	0.0100	
	2	0.0021	
	3	0.0400	0.0020
	4	0.0040	
	5	0.0029	
	6	0.0035	0.0100
Average		**0.0104**	**0.0060**
N12	1		
	2	0.0015	0.0003
	3	0.0009	
	4	0.0015	
	5	0.0015	0.0033
	6	0.0009	
Average		**0.0013**	**0.0018**
N16	1	0.0036	
	2	0.0100	0.0048
	3	0.0009	0.0010
	4	0.0030	
	5	0.0029	
	6	0.0015	
Average		**0.0037**	**0.0029**
N17	1	0.0043	0.0100
	2	0.0035	
	3	0.0040	0.0021
	4	0.0038	0.0027
	5	0.0045	
	6	0.0027	0.0025
Average		**0.0038**	**0.0043**
N21	1		0.0014
	2	0.0021	
	3	0.0012	
	4	0.0013	
	5	0.0005	0.0010
	6	0.0016	
Average		**0.0013**	**0.0012**

APPENDIX 13: Bud completely removed (treatment) and intact (control)

Variety	Replicate	Mass of larvae after feeding	
		Treatment	Control
NCo376	1	0.0200	0.0019
	2	0.0200	0.0200
	3	0.0200	0.0200
	4	0.0200	0.0100
	5	0.0200	0.0012
	6	0.0200	0.0100
Average		**0.0200**	**0.0105**
N11	1	0.0100	0.0022
	2	0.0100	0.0041
	3	0.0200	
	4		0.0016
	5	0.0100	
	6	0.0100	0.0100
Average		**0.0120**	**0.0045**
N12	1	0.0100	
	2	0.0029	0.0009
	3	0.0036	
	4	0.0007	
	5	0.0100	
	6	0.0003	0.0009
Average		**0.0046**	**0.0009**
N16	1	0.0100	
	2	0.0100	0.0041
	3	0.0100	0.0009
	4	0.0100	0.0100
	5	0.0100	0.0026
	6	0.0200	0.0011
Average		**0.0117**	**0.0037**
N17	1	0.0100	
	2	0.0100	0.0100
	3	0.0100	
	4		0.0013
	5	0.0100	
	6	0.0100	
Average		**0.0100**	**0.0057**
N21	1	0.0027	0.0012
	2	0.0100	0.0019
	3	0.0039	0.0100
	4	0.0100	0.0034
	5	0.0043	0.0100
	6		0.0100
Average		**0.0062**	**0.0061**

APPENDIX 14: Bud (treatment) and budscale (control) removed

Variety	Replicate	Mass of larvae after feeding	
		Treatment	Control
NCo376	1		0.0012
	2	0.0012	0.0019
	3	0.0019	0.0100
	4	0.0031	0.0034
	5	0.0007	0.0100
	6		0.0100
Average		**0.0017**	**0.0061**
N11	1	0.0018	0.0100
	2	0.0021	0.0020
	3	0.0100	0.0040
	4	0.0013	0.0042
	5		0.0100
	6		0.0035
Average		**0.0038**	**0.0056**
N12	1		0.0048
	2		0.0018
	3		0.0022
	4		0.0015
	5	0.0100	0.0015
	6	0.0100	0.0009
Average		**0.0100**	**0.0021**
N26	1		0.0022
	2	0.0100	0.0021
	3	0.0018	
	4	0.0031	0.0013
	5	0.0021	0.0005
	6	0.0015	0.0016
Average		**0.0037**	**0.0015**
N17	1		0.0036
	2	0.0100	0.0100
	3	0.0045	0.0009
	4	0.0048	0.0030
	5	0.0020	0.0029
	6		0.0015
Average		**0.0053**	**0.0037**
N21	1	0.0100	0.0043
	2	0.0045	0.0035
	3		0.0040
	4	0.0023	0.0038
	5		0.0045
	6		0.0027
Average		**0.0056**	**0.0038**

APPENDIX 15: Rind Hardness- using 2nd instar larvae

		Rind removed			Rind intact		
Variety	Replicate	Mass of larvae before	Mass of larvae after feeding	Mass gained/lost	Mass of larvae before	Mass of larvae after feeding	Mass gained/lost
NCo376	1	0.0167	0.0463	0.0296	0.0181	0.0415	0.0234
	2	0.0157	0.0602	0.0445	0.0135	0.0134	-0.0001
	3	0.0169	0.0326	0.0157	0.0230	0.0259	0.0029
	4	0.0269	0.0922	0.0653	0.0146		
	5	0.0087	0.0157	0.0070	0.0061		
	6	0.0107	0.0215	0.0108	0.0080		
N11	1	0.0138			0.0207		
	2	0.0141	0.0300	0.0159	0.0128		
	3	0.0169	0.1216	0.1047	0.0207		
	4	0.0201			0.0133	0.0461	0.0328
	5	0.0073			0.0065	0.0068	0.0003
	6	0.0110	0.0421	0.0311	0.0066		
N12	1	0.0228	0.0414	0.0186	0.0175	0.0268	0.0093
	2	0.0149	0.0379	0.0230	0.0148		
	3	0.0173			0.0180		
	4	0.0190	0.0176	-0.0014	0.0239		
	5	0.0130	0.0065	-0.0065	0.0074	0.0045	-0.0029
	6	0.0078	0.0147	0.0069	0.0147		
N26	1	0.0135			0.0157	0.0318	0.0161
	2	0.0192	0.0339	0.0147	0.0223	0.0516	0.0293
	3	0.0283	0.0332	0.0049	0.0189		
	4	0.0163			0.0164		
	5	0.0092			0.0041	0.0067	0.0026
	6	0.0070			0.0089	0.0165	0.0076
N17	1	0.0162			0.0195	0.0365	0.0170
	2	0.0218			0.0147	0.0263	0.0116
	3	0.0182			0.0166		
	4	0.0158			0.0190		
	5	0.0130			0.0064		
	6	0.0136			0.0063		
N21	1	0.0147			0.0217	0.0423	0.0206
	2	0.0178			0.0185	0.0264	0.0079
	3	0.0132			0.0241	0.0169	-0.0072
	4	0.0224			0.0240		
	5	0.0113			0.0050		
	6	0.0127			0.0061		

APPENDIX 16: Rind Hardness- using 3rd instar larvae

		Rind removed			Rind intact		
Variety	Replicate	Mass of larvae before	Mass of larvae after feeding	Mass gained/lost	Mass of larvae before	Mass of larvae after feeding	Mass gained/lost
NCo376	1	0.0934	0.1207	0.0273	0.0859	0.0847	-0.0012
	2	0.1606	0.1896	0.029	0.1105		
	3	0.0723	0.1096	0.0373	0.0641	0.055	-0.0091
	4	0.0705	0.1145	0.044	0.1145	0.1354	0.0209
	5	0.0962			0.1015		
	6	0.092	0.115	0.023	0.0828	0.0847	0.0019
N11	1	0.0814	0.1158	0.0344	0.0917		
	2	0.0995			0.0734	0.0938	0.0204
	3	0.0865	0.1091	0.0226	0.1278	0.0495	-0.0783
	4	0.0967	0.1372	0.0405	0.1145	0.5123	0.3978
	5	0.0789	0.1162	0.0373	0.0998	0.1342	0.0344
	6	0.1128			0.0623		
N12	1	0.1199	0.1228	0.0029	0.1381	0.0884	-0.0497
	2	0.12	0.1262	0.0062	0.0779	0.1489	0.071
	3	0.0936	0.1189	0.0253	0.0601		
	4	0.0672			0.0698	0.0901	0.0203
	5	0.1437			0.0899	0.0959	0.006
	6	0.1372			0.0576	0.0806	0.023
N26	1	0.1361	0.1471	0.011	0.1317	0.1222	-0.0095
	2	0.1541	0.1076	-0.0465	0.0984	0.0963	-0.0021
	3	0.1037	0.1281	0.0244	0.0749		
	4	0.0951	0.1228	0.0277	0.0882	0.162	0.0738
	5	0.1013			0.0902	0.1097	0.0195
	6	0.0674			0.123		
N17	1	0.0879	0.1291	0.0412	0.0775		
	2	0.108	0.1451	0.0371	0.078		
	3	0.0954	0.1046	0.0092	0.1197	0.1268	0.0071
	4	0.1081	0.1447	0.0366	0.0732		
	5	0.0871			0.0705	0.0779	0.0074
	6	0.0981			0.137		
N21	1	0.1022	0.1032	0.001	0.0822		
	2	0.1237	0.0862	-0.0375	0.1186	0.075	-0.0436
	3	0.1127	0.0879	-0.0248	0.0767	0.0345	-0.0422
	4	0.0988			0.1362	0.0849	-0.0513
	5	0.099	0.0869	-0.0121	0.0859		
	6	0.0803	0.1335	0.0532	0.0884		

APPENDIX 17: Mobility Experiments

				Position of recovery																				
Time	Variety	Plant	No. of eggs placed	Tot. no. of rec. larvae	BLB (abxl)	BLB (adxl)	BLB (edge)	BLS (abxl)	BLS (adxl)	BBd	BRP	BLSc	Int	MLB (abxl)	MLB (adxl)	MLB (edge)	MLS (abxl)	MLS (adxl)	MBd	MRP	MLSc	Int	GLS (ab)	Apex	Glue
24hrs (1day)	NCo376	1	319	166	1	65		52	16				8	5		5		1				11			12
	N11	1	296	217	7	34		45	44				15	17	15	14						3			10
	N12	1	303	135										30				15	18		10	4			58
	N17	1	305	156		111	30																		15
	N21	1	289	58		6								7											45
	N26	1	315	146		37	12		50				30												17
48hrs (2days)	NCo376	2	322	115					32	6	16	33						2							26
	N11	2	318	278						38		10	34					30	4	4	10	13			48
	N12	2	292	156								14	6												135
	N17	2	321	205		100		12		21			20					52							10
	N21	2	299	164			48						60												56
	N26	2	310	220	16		120						56					15							23
72hrs (3days)	NCo376	3	296	85		15		12					20					13			10				15
	N11	3	299	207					66	29	108														4
	N12	3	302	86								27													59
	N17	3	301	64						13			21												30
	N21	3	315	174		50							47												75
	N26	3	297	69						4		26										15			24
120hrs (5days)	NCo376	4	323	78			10				20	45								3					
	N11	4	293	136					2	9	7	23						3		3		11			78
	N12	4	304	86								15									45				26
	N17	4	306	85					3		5	37									32				8

	N21	4	303	106					10			30									10				56
	N26	4	298									78													20
168hrs (7days)	NCo376	5	309	89					13			40													36
	N11	5	301	162								58							15	5	71				13
	N12	5	298	120								25							10		5				80
	N17	5	288	75		4			5			2						3			21				40
	N21	5	305	74																	23				51
	N26	5	320	65								10									30				35
216hrs (9days)	NCo376	6	317	134								41									32				60
	N11	6	308	173								68							20	17	50				18
	N12	6	303	53								20									2				31
	N17	6	299	76								16								1	40				19
	N21	6	307	58		20							23												15
	N26	6	302	90		1						50	3								6				30

B: Bottom
LB: Leaf blade
LS: Leaf sheath
M: Middle
LSc: Leaf scar
Bd: Bud
RP: Root primordia
G: Green
Abxl: Abaxial (upper side)
Adxl: Adaxial (bottom side)