Genetic studies and recurrent selection for nematode resistance in maize

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General abstract

Plant-parasitic nematodes cause grain yield loss in maize. The most important genera of plant-parasitic nematodes demonstrated to be of economic importance to maize are *Pratylenchus* spp., *Meloidogyne* spp. and *Heterodera* spp. In Uganda, the most prevalent species are *Pratylenchus zeae* and *Meloidogyne* spp. The current study was initiated with the following objectives: (i) assessing farmers' awareness of maize nematodes, other maize production constraints, and desirable agronomic traits; (ii) assessing the efficiency of sterile carrot discs for mass culturing of *Pratylenchus zeae;* (iii) Characterising the inheritance of nematode resistance in maize, through, estimation of the general combining ability (GCA) of various parents, and the specific combining ability (SCA) of a parent in a cross with another parent; and through determining the contribution of cytoplasmic effects to inheritance of resistance to nematodes; and (iv) determine the level of nematode resistance among F_1 hybrids and estimate grain yield, heterosis and yield losses associated with maize hybrids under nematode infestation; (v) comparing the gains in nematode resistance and grain yield obtained following two cycles of S_1 progeny recurrent selection in three tropical maize populations.

In the participatory rural appraisal, data were collected from 120 households in two maize-growing districts. Maize roots and soil samples were also collected from farmers' fields, and nematode incidence determined. A small percentage (18.5%) of farmers was familiar with nematodes and the damage they cause in maize. *Pratylenchus zeae* occurred at generally higher frequencies than *Meloidogyne* spp. in susceptible cultivars. The landraces and the cultivar Longe 5 supported high nematode populations. Farmers also reported that Longe 5 had low yields when compared to the rest of the cultivars. Farmers' most preferred traits were pest and disease resistance, high grain palatability, long storage duration and large kernels. These findings justify the need for a programme to raise farmers' awareness on nematodes, their effects on crops as well as control strategies, and also a breeding programme that incorporates nematode resistance with farmer-preferred characteristics in maize.

Twenty live nematodes were transferred to the margins of each of the 40 sterile carrot discs contained in 3.5 cm diameter sterile glass Petri dishes. All cultures were maintained in the dark at 25 ± 1℃. The study revealed higher reprodu ction rates of *P. zeae* on carrot discs compared to excised maize roots. Each *P. zeae* inoculated on the carrot discs had reproduced 5,090 times after three months of incubation compared to a reproduction rate of 26.4 on excised maize roots. Carrot discs are therefore particularly useful for culturing *P. zeae*.

Thirty F_1 hybrids generated from a 6 x 6 diallel and two local checks were evaluated in three sites in an 8 x 4 alpha-lattice design in order to estimate GCA, SCA and genetic effects associated with nematode resistance in maize. The evaluations were done under nematode infestation and nematicide treated conditions. The nematode infested plots comprised an average Pi of 500 *P. zeae* and 100 *Meloidogyne* spp. per 100 g of soil per plot and lesser populations of other nematode species in the field trials. The GCA was more important for the reduction of *P. zeae* and *Meloidogyne* spp. densities and an increase in root mass, with a contribution of 72 to 93% of the phenotypic variance to these traits. Inbreds MP709 and CML206 had the highest GCA for *P. zeae* resistance, whereas for grain yield, CML444, CML312 and CML395 had the highest GCA. The SCA was important for heterosis in plant height and grain yield, contributing 43% and 58% of the phenotypic variance, respectively, under nematode infestation. Hybrids MP709/CML444 and MP709/CML395 had significant negative reciprocal effects for grain yield resulting from the negative maternal effects observed in parent MP709 when used as the female parent under nematode infestation. Using the graphical approach of the Hayman and Jinks analysis of genetic effects, overdominance gene action explained the non-additive variance observed for plant height, grain yield, number of root lesions, *P. zeae* and *Meloidogyne* spp. densities recorded under nematode infestation. Parents MP709, CML206, 5057 and CML444 contributed most of the dominant genes for *P. zeae* resistance. Parent CML444 contributed most of the dominant genes towards improved grain yield. The high GCA effects among some parents in the different sites suggest that breeding of widely adapted nematode resistant cultivars is possible. Whereas a ppreponderance of dominant genes and SCA effects would favour pedigree and various sib tests to improve grain yield under nematode pressure.

The 30 F_1 hybrids generated from the diallel cross were further assessed for nematode resistance, grain yield, heterosis and yield losses under nematode infestation and nematicide treated conditions. Results revealed more (24) *P. zeae* susceptible hybrids and a few (six) resistant hybrids. Grain yield across locations was higher by about 400 kg ha^{-1} under nematicide treated plots than under nematode infestation. Under both nematode infested and nematicide treated plots, the nematode resistant hybrids exhibited high yields ranging from 5.0 to 8.4 t ha^{-1} compared to 5.0 t ha^{-1} of the best check. Grain yield loss ranged between 1 and 28% among susceptible hybrids, and up to 12% among resistant hybrids, indicating that nematodes can cause economic yield losses especially when susceptible cultivars are grown. Under field conditions, favourable heterosis was recorded on 18 hybrids for *P. zeae*, and only on three hybrids for *Meloidogyne* spp. Under nematode infestation, only 16 hybrids had higher relative yield compared to the mean of both checks,

the best check and the trial mean, whereas it was 20 hybrids under nematicide treated plots. Hybrids CML312/CML206, CML444/CML395, CML395/CML444, CML444/CML312, CML312/CML444, CML395/CML312, CML312/CML395, CML312/5057, CML395/5057, 5057/CML444, 5057/CML206, CML395/MP709, CML444/MP709 had higher relative yield compared to the mean of both checks, the best check and the trial mean, both under nematode infestation and nematicide treatment, indicating stability of performance between stressed and non-stressed environment. In general, hybrids with the most outstanding performance under nematode infestation were CML395/MP709, CML312/5057, CML312/CML206, CML312/CML444, CML395/CML312 and CML312/CML395. Therefore, grain yield loss due to nematodes can be reduced by growing nematode resistant hybrids.

Two cycles of S_1 progeny recurrent selection were used to improve nematode resistance and grain yield of three tropical open pollinated varieties (Longe 1, Longe 4 and ZM521). The net gains in grain yield after the two cycles of selection were 6.3%, 10% and 22% for Longe 1, ZM521 and Longe 4, respectively. Each cycle of selection for nematode resistance improved grain yield by 200 to 600 kg ha^{-1} in the three maize populations. The damage caused by *P. zeae* reduced by 57%, 59% and 55%, and the *Meloidogyne* spp. by 65%, 39% and 59% for Longe 1, Longe 4 and ZM521, respectively, following the two cycles of selection. Realized heritability (h^2) for *P. zeae* and *Meloidogyne* spp. ranged from 66-96% at cycle 2. For grain yield, h^2 ranged from 80-86% at cycle 2. Broad sense heritability (H^2) for grain yield at cycle 2 ranged from 74-97% for the three maize populations. Therefore, the two cycles of S_1 progeny recurrent selection improved grain yield in the three maize populations through reduction of nematode densities.

Declaration

I, Frank Kagoda, declare that:

1. The research reported in this thesis, except where otherwise indicated, is my original research.

2. This thesis has not been submitted for any degree or examination at any other university.

3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

    a. Their words have been re-written but the general information attributed to them has been referenced

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5. This thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the thesis and in the references sections.

Signed

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Frank Kagoda

As the candidate's supervisors, we agree to the submission of this thesis:

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Dr. John Derera (Supervisor)

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Prof. Pangirayi Tongoona (Co-Supervisor)

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Dr. Daniel L. Coyne (Co-Supervisor)

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Dedication

This work is dedicated to my Dad, Mr. Christopher Kagoda and my guardian Fr. Francis Vester. These two gave me the foundation for furthering my education.

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List of abbreviations

a.i	Active ingredient
ANOVA	Analysis of variance
Ca	Calcium
CIMMYT	International Maize and Wheat Improvement Centre
CV(%)	Coefficient of variation
cv.	Cultivar
d.f	Degrees of freedom
ER	Ear rots
FAOSTAT	Food and Agriculture Organisation Statistics
frm	Fresh root mass
GLM	General linear model
h	Hour
h^2	narrow sense heritability
H^2	Broad sense heritability
ha	Hectares
IITA	International Institute of Tropical Agriculture
K	Potassium
kg	Kilograms
LSD	Least significant difference
m.a.s.l	Metres above sea level
Mg	Magnesium
MPH	Mid-parent heterosis
N	Nitrogen
NARO	National Agricultural Research Organisation
OM	Organic matter
P	Phosphorus
P_f	Final nematode population density (harvest)
P_i	Initial nematode population density (pre-sowing)
ppm	Parts per million
Proc	Procedure
R^2	Coefficient of determination
RF	Reproduction factor
RI	Resistance Index
s.e	standard error
S_1	Inbred line which has been selfed once

S_2	Inbred line which has been selfed in two successive generations
SAS	Statistical Analysis System
SPSS	Statistical Package for the Social Sciences
t	tonnes
tons	tonnes
Vr	Variance of family means
Wr	Covariance of family means
Wr+Vr	Parental order of dominance
Wr-Vr	Difference over arrays
δ^2	Variance

INTRODUCTION TO THESIS

1 Importance of maize

Maize *(Zea mays* L.) is one of the most important cereal crops in the world, essential for human diet and as a feed component for livestock (Pingali and Pandey, 2001). Global production is currently 817 110 509 tonnes, of which approximately 60% is produced in the developed countries, particularly in the USA; 27% is produced in China; and the rest is grown in countries of Latin America, Africa and southern Asia (Pingali and Pandey, 2001; FAOSTAT, 2009). Maize contributes approximately 30% of the calorific intake in East Africa, 50% in Southern Africa and 15% in West and Central Africa (Bänziger and Diallo, 2002).

In Sub-Saharan Africa, quantity of maize produced is 56 685 857 tonnes on an area of 30 269 406 ha (FAOSTAT, 2009). The bulk of this maize is produced by resource-limited, small-scale farmers under highly variable tropical and sub-tropical conditions (Bänziger and de Meyer, 2002), where average yields barely exceed 1.8 t ha^{-1}, much below the average of 5.1 t ha^{-1} for the rest of the world (FAOSTAT, 2009). Rosegrant *et al.* (2001) projected a two-fold increase in demand for maize in Sub-Saharan Africa by the year 2020, which implies an increased expansion of maize production into more marginal areas. This will attract increased risks of abiotic and biotic stresses hence minimal increases in production if yield potential is not maximized through additional resource inputs.

In Uganda, maize is a major staple crop providing over 40% of the population's calorie requirements with an annual consumption of about 23 kg per capita per year (NARO, 2002a). Maize also serves as an agricultural export crop to both rural and urban communities, with informal exports equivalent to 14-20% of total production (Magnay, 2004). Maize is largely produced on a low input/output system, with most areas of the country capable of producing two crops per year (Magnay, 2004). Major producing areas in Uganda are Iganga, Kumi, Sironko, Masindi, Kamwenge and Kapchorwa (NARO, 2002a). An average land area of 887,000 ha is currently under maize in Uganda; and production has averaged 1,272,000 tonnes with an average grain yield of 1.4 t ha^{-1} (FAOSTAT, 2009). Therefore, the growth in maize production has primarily been due to expansion of area planted than improvement in yield per hectare, as earlier observed by Kasenge *et al.* (2001). The stagnation or decline of maize grain yield has been attributed mainly to the extensive use of unimproved maize genotypes, depletion of soil fertility, erratic rainfall, prevalence of pests and diseases, little improvement in agronomic and post-harvest technologies and low levels of purchased inputs such as fertilizers and other agrochemicals (Sserunkuuma *et al.*, 2001).

2 Maize production constraints in Uganda

Major maize production constraints in Uganda include diseases, nutrient deficiencies and pests. Persistent foliar diseases in Uganda include grey leafspot (GLS), Turcicum leaf blight (TLB), and maize streak virus (MSV) (Bigirwa *et al.*, 2001). *Sternocarpella maydis*, *Fusarium graminearum* and *F. verticillioides* have been associated with ear rots, stalk rots, and seedling blights on maize in Uganda (Bigirwa *et al.*, 2005). The maize plant requires high levels of nitrogen (N), potassium (K), phosphorus (P), calcium (Ca) and magnesium (Mg) for maximum yield (Kochhar, 1981). However, less than 3.1% of farmers use inorganic fertilizer, manure and compost on maize in Uganda (Sserunkuuma, 2002). Pests of maize in Uganda include the maize leaf hopper (*Cicadulina mbila*), stem borers (stalk borers), aphids, weevils, termites, striga weed and nematodes (Kalule *et al.*, 1997; NARO, 2002b; Butseya *et al.*, 2005; Talwana *et al.*, 2008; Kagoda *et al.*, 2010).

Plant-parasitic nematodes are among the most neglected parasites in terms of research yet they cause considerable loss in maize production during crop development. Nematodes also aggravate plant damage under moisture and nutrient stressed conditions in particular (McDonald and Nicol, 2005). However, yield losses often go unnoticed due to lack of typical symptoms (Asmus *et al.*, 2000). Quantification of grain yield losses associated with nematodes may also be affected by specific nematode extractions (Riekert, 1995) and resistance assessment methods (Ibrahim *et al.*, 1993). Despite these shortcomings, maize remains prominent in many local economies, and is extensively used in rotation systems hence knowledge of its host status to economically important nematode species and how to avert them is crucial.

Yield losses in maize due to nematode infestation have been recorded in some countries in Africa. Research in Kenya has showed 50% yield loss from nematodes, with the lesion nematodes rated as the most problematic (Bridge, 1994; Kimenju *et al.,* 1998). In South Africa, the estimated annual maize yield loss to nematode infestation is 12% (Keetch, 1989) with *Meloidogyne javanica* and *M. incognita* being the most common root-knot nematode species (Riekert, 1996). In USA, Johnson (1975) reported nematicide application to have increased yield of sweet corn by 31% compared to the controls. According to Caveness (1992) and Jones and Perry (2004), over 60 nematode species, of which *Pratylenchus zeae* is the most abundant, are associated with maize in different parts of the world, and yield losses due to their attack ranges from 10 – 80%. Afolami and Fawole (1991) reported maize grain yield reduction as high as 50% under continuous cropping system as a result of *P. sefaensis* parasitism.

In Uganda, however, no efforts towards documenting yield losses associated with nematode infestation have been made. Nevertheless, studies by Butseya *et al.* (2005) associated 22 species of plant-parasitic nematodes from 10 genera on cereals, namely maize (Zea mays), sorghum (*Sorghum bicolor*), wheat (*Triticum aestivum*), barley (*Hordeum vulgare*) and finger millet (*Eleusine corocana*), in Uganda. Fifteen of these genera were recovered from maize roots. The most frequently encountered nematodes included species of *Pratylenchus*, *Ditylenchus*, *Helicotylenchus, Scutellonema, Meloidogyne* and *Aphelenchoides*.

3 Justification of the current study

Resource-limited, small-scale farmers account for the largest share of maize production in Uganda. The majority rely on unimproved cultivars despite the availability of improved cultivars, which would provide better returns. The major reasons why farmers do not use improved maize cultivars is lack of awareness, high purchase cost, low palatability, and expense of production (Sserunkuuma, 2003). Farmers are therefore faced with low grain yields, fluctuating between 0.8-1.5 t ha^{-1} (Kasenge *et al.*, 2001; NARO, 2002a; FAOSTAT, 2007; FAOSTAT, 2009). In order to enhance adoption of new cultivars, farmers need to be engaged early in the variety development process (Witcombe *et al.*, 1996). Therefore, participatory rural appraisals and participatory breeding strategies before commencing breeding programmes would therefore be of great potential benefit.

Some of the composite cultivars (e.g. Longe 1, Longe 4, and Longe 5) available in Uganda high are high yielding (4-5 t ha^{-1}) but only under well managed conditions at the research stations (NARO, 2002a). Also, hybrids, such as Longe 3H, Longe 6H, Longe 7H, Longe 8H, PAN 67, SC 627 and SC 407 have provided even higher yields (8 t ha^{-1}), but still under research conditions (NARO, 2002a). According to Imanywoha *et al.* (2005), most of these improved cultivars have high protein content, and provide acceptable levels of resistance to key biotic stresses such as TLB, MSV, GLS, ER, and weevils. Growing these improved cultivars has also enhanced nitrogen utilization efficiency and drought resistance (Imanywoha *et al.*, 2005). However, breeding initiatives that resulted in the generation of these cultivars did not attempt to incorporate nematode tolerance as a control option against nematodes. Some of these cultivars are therefore prone to nematode attack (Talwana *et al.*, 2008; Kagoda *et al.*, 2010) yet the effects of nematodes have always been underestimated not only by breeders but also by agronomists, pest management consultants, and farmers (McDonald and Nicol, 2005). This indicates that awareness of the potential of nematodes to cause yield loss in maize is still low in most communities in Uganda.

Similarly, few research stations in developing countries have screening facilities for nematodes, and trained personnel. For example, in Uganda, only one fully-fledged laboratory exists, which does screening and identification of nematodes. Raising inoculum for some nematode species is cumbersome, which might be one of the reasons for the laxity in nematode research on maize. For example, *P. zeae*, a major nematode of maize in Uganda, has for long been cultured on maize roots (Meyer, 1984), which happens to be a very slow yet expensive process to raise inoculum. Therefore, in the current study, efficiency of carrot discs as media for raising *P. zeae* inoculum was studied.

An 'improved maize cultivar' which is susceptible to nematodes will definitely have damaged, inefficient and impaired root systems that are less able to take up water, applied N and other nutrients once grown in nematode infested soils ([1]Coyne, D., personal communication). Such a cultivar can never reach its yield potential under farmers' conditions. Similarly, De Waele and Elsen (2002) reported root tissue reduction due to nematode infection to lead to a reduced uptake of water and nutrients by the plant (De Waele and Elsen, 2002). According to Whitehead (1998), an estimated 10% of the world crop production is lost as a result of plant nematode damage (Whitehead, 1998). An estimated yield loss due to nematodes of 78–125 billion US dollars world-wide annually has been reported (Sasser and Freckman, 1987). There is hence a need to devise strategies aimed at minimising yield loss resulting from nematode attack. For nematode control, host resistance is advocated because it is cost effective and poses no technical difficulties to the farmer, provided that resistance genes are readily available (Trudgill, 1991). Similarly, though nematode control options such as the use of nematicides, crop rotation, and bare fallow are effective, they are often inappropriate on low value field crops such as maize (Sikora, 1992). A strategy to develop maize genotypes with acceptable levels of resistance to nematodes will require a clear understanding of the nature of inheritance of the resistance genes and the establishment of an appropriate breeding strategy for nematode resistance in maize.

4 Objectives of the study

The choice of objectives was dependant on achieving the overall research goal of contributing to increased maize production, especially among small-scale farmers in Uganda, through production of hybrids and OPVs with resistance to the major nematode pests of maize in Uganda, yet retaining all the other desirable attributes. The overall objective was to understand farmers' awareness of nematodes, the related damage and farmers' desirable attributes in maize, coupled with understanding the genetics of nematode

[1] Dr. D.L. Coyne, Nematologist, IITA-Tanzania, ARI-Mikocheni, Dar es Salaam

resistance in maize, and yield losses associated with nematode damage. The specific objectives were to:

i) Assess farmers' awareness of maize nematodes, other maize production constraints, and desirable agronomic traits;
ii) Assess the efficiency of sterile carrot discs for mass culturing of *Pratylenchus zeae;*
iii) Characterise the inheritance of nematode resistance in maize, through:
   - Estimating the general combining ability (GCA) of various parents, and the specific combining ability (SCA) of a parent in a cross with another parent;
   - Determining the contribution of cytoplasmic effects to inheritance of resistance to nematodes;
   - Estimating the genetic effects which control the inheritance of nematode resistance in maize;
v) Determine the level of nematode resistance among F_1 hybrids and estimate grain yield, heterosis and yield losses associated with maize hybrids under nematode infestation;
vi) Compare the gains in nematode resistance and the resulting grain yield obtained following two cycles of recurrent selection in three tropical maize populations.

5 Research hypotheses

The following hypotheses were tested:

i. Farmers are aware of the dangers of plant-parasitic nematodes and other constraints limiting maize production in their localities and have specific preferences for agronomic traits;
ii. Sterile carrot discs offer a better and relatively less laborious alternative for rearing *P. zeae*;
iii. Maize inbred lines have good GCA associated with nematode resistance and high grain yield;
iv. Maternal effects are important in the inheritance of resistance to nematodes in maize;
v. Adequate genetic variations, both additive and dominance, are involved in conditioning resistance to nematodes in maize;
vi. Variation in nematode resistance exist among maize hybrids;
vii. Hybrid vigour towards nematode resistance and grain yield can be obtained among nematode resistant maize hybrids;
viii. Plant-parasitic nematodes, if left uncontrolled, lead to grain yield loss among susceptible maize hybrids;
ix. Genetic gain in nematode resistance is achievable through two cycles of S_1 recurrent selection, and a significant increase in grain yield could be realised.

6 Structure of the thesis

This thesis is made up of eight main sections that include six chapters as shown below:

1. Introduction to thesis
2. Chapter One: Literature review
3. Chapter Two: Awareness of plant-parasitic nematodes and maize varietal preferences by smallholder farmers in East and Southern Uganda: A basis for assessing nematode resistance breeding needs in African maize
4. Chapter Three: Monoxenic culture of *Pratylenchus zeae* on carrot discs
6. Chapter Four: Genetic analysis of resistance to nematodes in maize (*Zea mays* L.)
7. Chapter Five: Grain yield and heterosis of maize hybrids under nematode infested and nematicide treated conditions
5. Chapter Six: Response to two cycles of S_1 progeny recurrent selection for nematode resistance in three tropical maize populations
8. General overview of the study.

All chapters except chapter one (literature review) are written in IMRAD format that include Introduction, Materials and methods, Results, and Discussion. All chapters have a reference list, and contain some limited repetition and overlap of content.

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CHAPTER ONE

Literature review

1.1 Introduction

This chapter provides an insight into the research by (a) reviewing nematodes of maize, with emphasis on *Pratylenchus* zeae and *Meloidogyne* species, the most prevalent in Uganda; examining the morphology, biology, life cycle, distribution, economic importance and damage symptoms caused by these nematodes; (b) examining the different nematode management options; (c) reviewing the breeding strategies for resistance to maize nematodes, particularly *Pratylenchus* zeae and *Meloidogyne* spp.; (d) examining the different techniques for rearing *Meloidogyne* and *Pratylenchus* spp.; e) reviewing recurrent selection and its applicability to maize; (f) examining the role of participatory rural appraisal in plant breeding. Conclusions drawn from the review are provided at the end of the chapter.

1.2 Nematodes of maize

Over 60 nematode species have been associated with maize across the world (Jones and Perry, 2004; McDonald and Nicol, 2005). The most important groups of plant-parasitic nematodes demonstrated to be of economic importance to maize production are: (i) the root-knot nematodes, *Meloidogyne* spp.; (ii) the root lesion nematodes, *Pratylenchus* spp.; and (iii) the cyst nematodes, *Heterodera* spp. (McDonald and Nicol, 2005). In Uganda, the most prevalent nematode species on maize are *Pratylenchus* spp. and *Meloidogyne* spp. (Butseya *et al.*, 2005; Talwana *et al.*, 2008; Kagoda *et al.*, 2010).

1.2.1 Genus *Pratylenchus* Filipjev, 1936

Pratylenchus species belong to the order Tylenchida, suborder Tylenchina, superfamily Tylenchoidea and family Pratylenchidae (Heyns, 1971; Dropkin, 1989; Luc *et al.*, 2005). They are obligate plant parasites, living as migratory endoparasites in underground parts of the plant. Hunt *et al*. (2005) described *Pratylenchus* spp. as small nematodes (<1mm long), which die curved ventrally on application of gentle heat. According to Dropkin (1989), *Pratylenchus* spp. are elongate, from 340 to 800 µm long, with a length/width ratio of 15 - 35. Under a stereomicroscope, they are recognizable by a flat head; strong cephalic framework; a short, thick stylet, about 14-20 µm long, with prominent rounded anteriorly concave basal knobs (Fig. 1.1). They have no marked sexual dimorphism in the anterior region. The labial region is further divided into 2, 3 or 4 annules, which are continuous with the body contour.

The oesophagus is equally developed in both sexes, with oesophageal gland lobes overlapping the intestines ventrally (Saddiqi, 1972a; Dropkin, 1989).

The females are characterized by a well developed posterior vulva at 70-80% of body length; a genital system with a single, anteriorly directed tract (monoprodelphic); and a variable post-vulval section, which may show some differentiation but which is never functional (Saddiqi, 1972b). The females also have an oval or round spermatheca, usually filled with sperm in the bisexual species. The tail is broadly rounded or pointed, constituting 3.5-9% of the body length, with a truncate terminus, which may be smooth or annulated. The male *Pratylenchus* spp. are characterized by: short, dorsally convex-conoid tails; bursa extending to tail tip; slender and arcuate spicules (Saddiqi, 1972b). In some species, males are absent but where present, are smaller than the females.

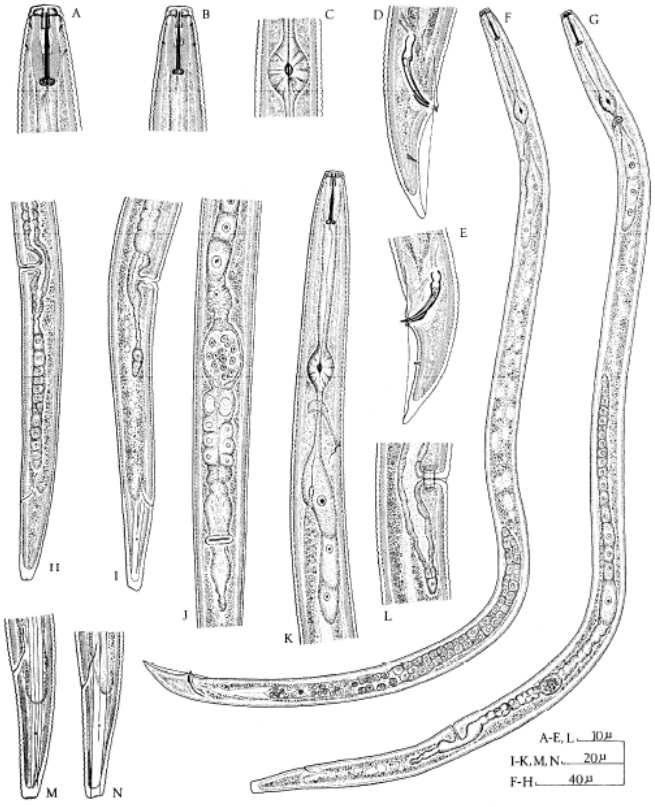

Figure 1.1: *Pratylenchus* spp. A, B, head end; C, median bulb; D, E, male tail; F, G, entire male and female; H, I, female posterior region; I, ventral view of vulva and genital tract; K, oesophagus; L, postuterine sac; M, N, female tail (Saddiqi, 1972a)

Pratylenchus spp. are migratory endoparasites with all growth stages found in the root cortex (Loof, 1978; Hunt *et al.*, 2005). They feed on cortex cells and form cavities containing colonies of nematodes of all stages. Reproduction is by both sexual and parthenogenetic means (Loof, 1978). In contrast to many other nematode species, *Pratylenchus* spp. reproduce more actively in roots of plants under stress (Dropkin, 1989). Eggs, from which

second-stage juveniles hatch, are deposited in clusters within roots or in soil (Dropkin, 1989). All stages move between soil and roots, preferring mainly coarse textured, sandy soils. The life cycle is completed in 3 - 4 weeks and the nematodes are capable of surviving in the absence of host plants for several months (Dropkin, 1989). At low soil moistures, some species survive well for more than a year in the absence of host crops. Major species include *P. brachyurus, P. coffeae, P. goodeyi, P. penetrans* and *P. zeae* (Dropkin, 1989). Most of these species are viewed as polyphagous, except *P. goodeyi*, which was previously reported as restricted to banana (Hunt *et al.*, 2005), but has since been recorded on maize in Uganda (Butseya *et al.*, 2005; Talwana *et al.*, 2008). On maize, Todd and Oakley (1996) observed stable or declining populations of *Pratylenchus* spp. in seminal roots as opposed to increasing numbers in adventitious roots as the season progressed. However, nematode density was generally higher in the seminal roots than the adventitious roots. In the presence of maize, *Pratylenchus zeae* migration was greatest in a sandy loam soil, intermediate in loam, and least in clay (Endo, 1959; Norton, 1978a). Maize has been particularly found to favour *P. zeae* growth and suppress *Trichodorus christiei, Helicotylenchus dihystera*, and *Xiphinema americanum* in a 6-year rotation in Georgia (Brodie *et al.*, 1969).

The most commonly encountered *Pratylenchus* species in sub-tropical and tropical regions are *P. zeae, P. brachyurus* and *P. penetrans* (De Waele and Jordaan, 1988; Jordaan *et al.*, 1989; Lordello *et al.*, 1992; Hunt *et al.*, 2005). Infrequently encountered species include *P. coffeae, P. delattrei, P. goodeyi* (Prasad *et al.*, 1995), *P. hexincisus, P. neglectus, P. pratensis, P. scribneri* and *P. thornei* (Loof, 1978). *Pratylenchus penetrans* is mainly found in the cooler regions of the tropics whereas *P. goodeyi* can be found on banana (*Musa* spp.) in Ethiopia, Kenya, Tanzania, Uganda, Crete, the Canary Islands and Australia (Hunt *et al.*, 2005). The *P. hexincisus* damage is more associated with dryland maize whereas *P. scribneri* damage is significant in irrigated maize (Smolik and Evenson, 1987). Distribution of lesion nematodes in maize fields can be greatly influenced by their wide host range both on other crops (Loof, 1978) and weeds, which greatly affects selection of crops for rotation (Egunjobi, 1974; Jordaan and De Waele, 1988). Some of the other crops on which *Pratylenchus* spp. has been identified include potatoes (*Solanum tuberosum*), orchards, pineapples (*Ananas sativus*), tobacco (*Nicotiana tabacum*) and pearl millet (*Pennisetum glaucum*) (Heyns, 1971).

Lesion nematodes are widely distributed in maize fields, and have been associated with poor growth and yield reduction (Dickson and McSorley, 1990; Afolami and Fawole, 1991; McDonald and Van Den Berg, 1993). Damage becomes more pronounced under a continuous maize cropping system (Reversat and Germani, 1985; Maqbool and Hasmi,

1986). However, yield loss estimates in maize due to *Pratylenchus* spp. are scarce, mostly as a result of the confounding effects of other factors (Dickson and McSorley, 1990; Todd and Oakley, 1996; Koenning *et al.*, 1999). Nevertheless, in Nigeria, *P. brachyurus* has been reported to be responsible for 28.5% yield reduction for every 50% increase in nematode density (Egunjobi, 1974). Using regression analyses, Todd and Oakley (1996) reported a 1% loss in maize seed test mass for each 10-fold increase in nematode density and a 1% loss in seed yield for each 1 000 nematodes/g root.

Following treatment with nematicides, maize yield increases have been reported, suggesting that lesion nematodes are important limiting factors in maize cultivation (El-Meleigi, 1989; Riekert, 1996a). For example, Walter (1979) observed yield increases of 33 - 128% in South Africa following the application of nematicides. In the USA, a yield increase of 10 - 54% was reported following application of a nematicide (Bergeson, 1978; Norton *et al.*, 1978). Secondary infections, following nematode damage, by fungi and bacteria are, however, a major obstacle in making precise evaluations of losses in maize due to lesion nematodes (Egunjobi, 1974). In fact, combined inoculations of *P. zeae, P. brachyurus* and *Fusarium moniliforme* on maize constrain plant growth, especially at the seedling stage, more than inoculation with nematodes alone and *F. moniliforme* alone (Jordaan *et al.*, 1987). Highly significant negative relationships have, however, been reported between *P. zeae* and *P. brachyurus* and between *P. brachyurus* and *P. penetrans* probably because *P. brachyurus* prefers soils with a higher clay content (Jordaan *et al.*, 1989).

Generally, the nematode species, population density and environmental conditions affect symptom expression on nematode infested maize (McDonald and Nicol, 2005). Therefore, above-ground symptoms are not highly specific (Jepson, 1987). They, however, include wilting, leaf chlorosis, dieback and stunted growth (Dropkin, 1989). *Pratylenchus brachyurus* has been particularly associated with reductions in maize and sorghum shoot weight (McDonald and Van Den Berg, 1993). *Pratylenchus* spp. damage causes discolouration of affected root tissues, which are observed as brownish necrotic lesions on the cortex of roots as a result of nematode feeding, hence the name “root lesion nematode”. It is actually possible to diagnose damage due to lesion nematodes based on the presence of small lesions on the root surface of the affected plant (Corbett, 1976; Fortuner, 1976). According to Gao and Cheng (1992), nematode damage to fibrous root systems results in destruction of the cortical parenchyma and epidermis, which may cause sloughing-off of the tissue and severe necrosis. This may be followed by severe root pruning as well as proliferation of lateral roots (Ogiga and Estey, 1975; Zirakparvar, 1980). *Pratylenchus zeae* particularly causes a mechanical breakdown of cells and necrosis of stellar and cortical tissues, resulting

in formation of cavities (Olowe and Corbett, 1976; Olowe, 1977). Studies carried out in South Africa on maize and sorghum revealed significant reductions in root mass due to *P. zeae* and *P. brachyurus* infestation (McDonald and Van Den Berg, 1993). Patel *et al.* (2002) recorded considerable reduction in root and shoot mass, plant height and chlorophyll content and an almost ten-fold increase in *P. zeae* numbers in maize grown in pots. Corbett (1976) reported *P. brachyurus* to cause even more necrosis than mechanical damage.

Pratylenchus zeae Graham, 1951 is a pest of several crops, some of these are: maize, tobacco, cotton (*Gossypium* spp.), sugarcane (*Saccharum officinarum*), sorghum (*Sorghum bicolor*), finger millet (*Eleusine corocana*), rye (*Secale cereal*), soybean (*Glycine max*), tomato (*Lycopersicum esculentum*), sweetpotato (*Ipomoea batatas*), wheat (*Triticum* spp.), peanut (*Arachis hypogaea*), barley (*Hordeum vulgare*), and cowpea (*Vigna unguiculata*) (Fortuner, 1976). Some of the countries where this nematode was first recorded on maize include USA, Rhodesia (currently Zimbabwe and Zambia) and Malawi. *Pratylenchus zeae* is a migratory endoparasite of the root cortex, which enters the smaller roots at any point (Fortuner, 1976). All stages are found in the outer parenchyma cells, never in the vascular tissues, usually lying parallel to the root axis (Fortuner, 1976). The systematic position of *Pratylenchus zeae* is as follows: Order Tylenchida, Super Family Tylenchoidea, Family Pratylenchidae; Sub Family Pratylenchinae; genus *Pratylenchus*; species *zeae* (Heyns, 1971). The female *P. zeae* as described by Fortuner (1976) and Loof (1978) has a slender body, almost straight when relaxed, and marked by very faint annules (Fig. 1.2). The lip region is not set off from body and bears three annules. The heavily sclerotized labial framework is not extended backwards laterally. The spear or stylet is 15-17 µ long, with broad anteriorly flattened basal knobs. The oviduct is indistinct and the uterus is short. The spermatheca is small and round without sperms. The vulva is located at 68-76% of the body; it has a narrowly rounded smooth tail tip. The tail is tapering with 16-25 annules.

Male *P. zeae,* as described by Fortuner (1976), are extremely rare, and if present, do not have a spermatheca. They are not essential for reproduction. They have slender spicules, ventrally arcuate, 14-15µ long. They have faintly crenate bursa margins (Fortuner, 1976; Loof, 1978). Males are similar to females in morphology except for sexual dimorphism (Fortuner, 1976).

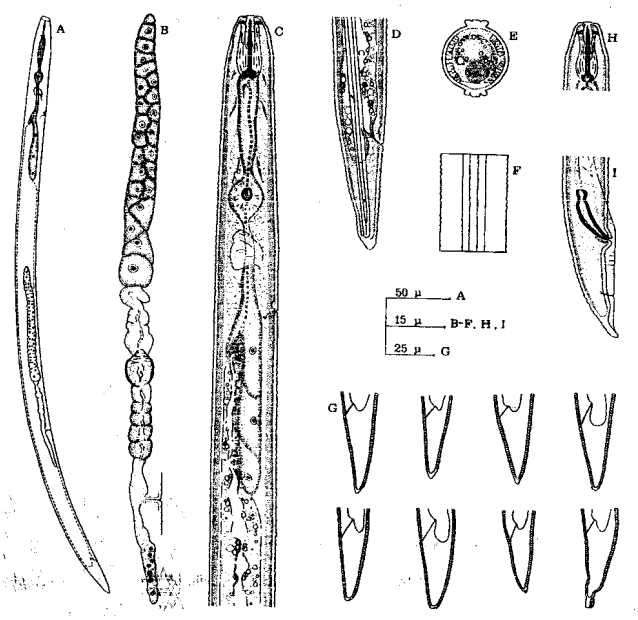

Figure 1.2 Pratylenchus zeae: Graham. A-G. Female. A. Entire female. B. Ovary. C. Oesophageal region. D. Tail. E. Mid-body transverse section showing lateral fields. F. Lateral field in surface view. G. Tails. H. Head of male. I. Tail of male. (Fortuner, 1976).

Pratylenchus zeae lays relatively few eggs either singly or in scattered groups of 3-4 within a single lesion (Fortuner, 1976). Hatching takes 15-20 days, and the period from egg to maturity is 35-40 days. In the USA, overwintering occurs in dead roots of crabgrass (*Digitaria* spp.), maize, cotton and, to a lesser extent, tobacco (Fortuner, 1976). Nematodes are also able to survive the winter in soils without roots. Cellulolitic enzymes were found to be present in the homogenates and extracts of *P. zeae*, which probably helps the nematode to penetrate cell walls (Fortuner, 1976). There is little or no migration in the absence of roots (Loof, 1978).

1.2.2 Genus *Meloidogyne* Goeldi, 1887

Root-knot nematodes are obligate plant parasites consisting of more than 50 species world-wide (Sasser, 1977). Within species, several races with differential host ranges occur (Sasser and Triantaphyllou, 1977; Kleynhans, 1991). They belong to the order Tylenchida and family Heteroderidae (Heyns, 1971; Dropkin, 1989). The young vermiform larvae penetrate the roots of plants near the growing points, migrate towards the stele and become sessile inside the root as adult females.

Meloidogyne spp. comprises of adult females, adult males and juveniles (Fig. 1.3). Adult females are flask-shaped, sedentary nematodes, embedded inside the root (Dropkin, 1989). The adult females are about 0.5 mm long and 0.3-0.4 mm wide. They are characterized by a

distinctive pattern of striations surrounding the vulva and anus (perineal pattern), which is used for species identification (Heyns, 1971; Dropkin, 1989). The adult males are elongate and move about slowly in the soil/root. They vary in length, with a maximum of up to 2 mm, and with a length/width ratio close to 45. The head is not offset, and the stylet is almost twice as long as that of the female (Heyns, 1971; Dropkin, 1989). The male tail is short and rounded. Juveniles resemble those of *Heterodera* except that they are more delicate, with a shorter and thinner stylet (Heyns, 1971).

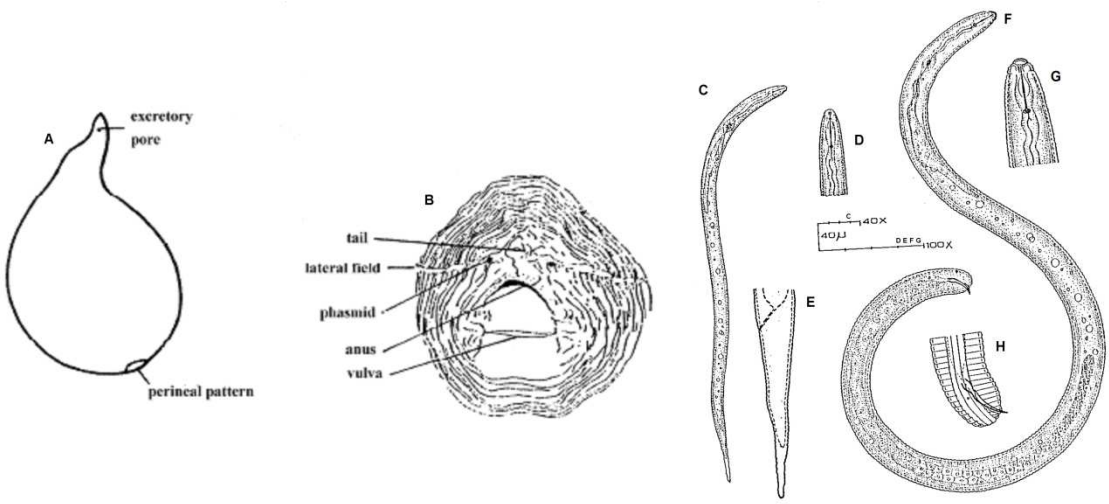

Figure 1.3: *Meloidogyne* spp. A-G. A. Entire female body. B. Perineal pattern. C. Second stage larva. D. Anterior end of larvae. E. Larval tail. F. Entire male body. G. Male head. H. Male tail. (Williams, 1972).

According to Dropkin (1989), most *Meloidogyne* spp. reproduce without males though both sexes are necessary in other species. Eggs are deposited into a gelatinous egg sac that probably protects them from desiccation and perhaps from micro-organisms. In most host-parasite combinations, a gall is formed from which an egg sac usually protrudes. The embryo develops into a juvenile that moults once within the egg. Second-stage juveniles hatch under favourable temperature and moisture conditions and move through soil towards growing root tips. Once within a root, a juvenile moves between cells until it locates a site close to the stele, often in the area of a developing side root (Dropkin, 1989). Here, it becomes sedentary and causes the transformation of cells upon which it feeds. The juvenile swells, moults rapidly a second and third time without feeding, and matures into an adult male or female (Dropkin, 1989). Adult males, which may aggregate within a single egg sac, elongate within the fourth-stage cuticle and emerge from roots. The adult female remains attached to its feeding site within the stele and with its posterior at the root surface (Dropkin, 1989). It continues to produce eggs throughout its life, sometimes reaching a total of up to 1 000 eggs (Barker *et al.*, 1985; Dropkin, 1989). The life cycle may be as short as 3 weeks and as long as several months depending on the host and temperature (Taylor and Sasser,

1978). The sex ratio is also influenced by the environment, with more males developing when roots are heavily attacked or nutrition is inadequate (Dropkin, 1989).

Histologically, *M. javanica* infection of maize roots shows typical multinucleated giant cell development in vascular tissue as well as embedded egg masses in inconspicuous galls, mostly close to root apexes (Asmus *et al.*, 2000). Staining of maize root systems is advisable to be able to assess nematode penetration if root-knot nematodes are suspected or juveniles are detected in the soil (McDonald and Nicol, 2005). Root tip galls can, however, be confused with galls produced by ectoparasites like *Xiphinema*. Riekert (1995) modified the sodium hypochlorite (NaOCl) extraction technique specifically for root-knot nematode assessment on maize though gall indices and other staining methods can still be used.

Root-knot nematodes are widely distributed and are of economic importance on most crops in the world including weeds (Sasser, 1977; Meyer and Van Wyk, 1989). On maize, several species of *Meloidogyne* have been reported from around the world. *Meloidogyne incognita* and *M. javanica* have been reported to damage maize in almost all maize-growing regions of the world (McDonald and Nicol, 2005). *Meloidogyne africana* and *M. arenaria* have been recorded on maize in India (Krishnamurphy and Elias, 1967) and Pakistan (Maqbool, 1980). *Meloidogyne arenaria* has been reported in the USA on maize (Ibrahim *et al.*, 1993). However, despite the occurrence of root-knot nematodes in maize fields, information on their importance and the economic losses they cause is scarce. According to Dickson and McSorley (1990), failure for maize to exhibit yield reduction due to nematode parasitism is a result of extensive root growth in this crop after the seedling stage. This results from the high fertilization and irrigation levels applied to this crop in commercial settings, hence obscuring measurable injury levels. Nonetheless, it is important to be alert to root-knot nematode infestation of maize, particularly in low input production conditions.

Above-ground symptoms of *Meloidogyne* spp. damage on maize include stunting, leaf chlorosis, wilting and patchy growth. Root galls may be small or large, terminal or sub-terminal or further back along the root (McDonald and Nicol, 2005). Typical gall symptoms may be totally absent (Idowu, 1981; Riekert, 1995; Asmus *et al.*, 2000), leading to maize being mistakenly considered a poor host or even immune to root-knot nematodes. However, according to Koenning *et al.* (1999), lack of adequate nematode control measures on maize has been the major reason for ignorance of nematode damage on this crop. In Jamaica, greater root-knot damage occurred when maize was sown after sugarcane (Hutton, 1976). Studies on interaction between *M. incognita* and mosaic virus showed that nematode

reproduction was greater when both pathogens were together than when alone (Goswami and Raychaudhuri, 1978).

1.2.3 Other nematodes associated with maize

Besides *Pratylenchus* and *Meloidogyne* species, other nematodes associated with maize are *Heterodera, Belonolaimus, Criconemella, Hoplolaimus, Tylenchorhynchus, Helicotylenchus, Rotylenchulus, Longidorus, Paratrichodorus*, *Ditylenchus, Quinisulcius* and *Radopholus* species (McDonald and Nicol, 2005). Among these, *Heterodera* spp. is more widely spread on maize in sub-tropical and tropical countries, with the most important species being *H. zeae, H. avenae* and *Punctodera chalcoensis* (Luc, 1986). In Uganda, Butseya *et al.* (2005) recorded *Ditylenchus*, *Helicotylenchus, Scutellonema* and *Aphelenchoides* as the most prevalent species after *Pratylenchus* and *Meloidogyne*. Among these, *D. dipsaci* has been reported to cause seedling yield losses and to affect rate of seedling development elsewhere (Knuth, 2000). Seed-borne specimens of *D. dipsaci* and *Ditylenchus* spp. juveniles have also been detected on maize (Tenente *et al.*, 2000), and associated with plant lodging. Generally, nematode species and associated damage in Africa have been little documented in relation to Europe and the USA. Other nematode problems may therefore arise as time passes and as information accumulates.

1.3 Nematode management options in maize

Nematodes are carried by soil, water, wind and plants, making it virtually impossible to prevent dispersal from infested fields. In each season, crops provide abundant, closely spaced roots, well supplied with water and nutrients, which is a perfect environment for rapid nematode reproduction. Parasitic nematodes like *Pratylenchus* spp. have been reported to survive on remnants of old maize roots, weeds and soil during the dry season, from which they spread rapidly during the rainy season (Egunjobi and Bolaji, 1979). Therefore, nematode control is now considered part of good farming practices in many parts of the world, but primarily in developed countries (Dropkin, 1989). The different nematode management practices, as applied on maize, include use of chemicals, cultural practices, biological control and host plant resistance (HPR).

1.3.1 Chemical control

Nematicides are effective in controlling nematodes on maize but their utilization is limited mainly for economic and health reasons, and the fact that results have been inconsistent (McDonald *et al.*, 1987; Riekert, 1996b). Effective nematode control in maize has been achieved with nematicides such as oxamyl (Vydate), aldicarb (Temik 150G^{TM}) and fenamiphos (Nemacur) (Scholte and s'Jacob, 1983; Riekert, 1996a; Johnson *et al.*, 1999).

Aldicarb has also been reported to be effective in control of *Pratylenchus neglectus* and *P. thornei* when applied at a rate of ≥ 2.5 kg a.i ha^{-1} on wheat and barley (Taylor *et al.*, 1999).

According to Rhoades (1979), soil fumigants and non-volatile nematicides increased growth and yield of field maize compared to untreated controls in Florida. Actual yield increases were 28% in the first year to 58% in the second year of nematicide application, and were related to the control of sting nematodes (*Belonolaimus longicaudatus*). Johnson and Dickson (1973) reported that *B. longicaudatus* could economically be controlled in maize with low rates of several organophosphates and carbamate nematicides incorporated in 38 cm band row treatments just prior to planting. Rhoades (1979) affirmed that application of fenamiphos, carbofuran, aldicarb, and oxamyl in a 38 cm band incorporated with rotary wheels at a rate of 2.2 a.i kg ha^{-1} just prior to planting controlled *B. longicaudatus* better than when the chemicals were applied in a 25 cm band in front of the press wheel or in the seed furrow with the planter. Increased plant growth and vigour were observed within a few days after plant emergence in plots treated with nematicides. The nematicide treated plots had dark green, vigorous plants with thick stalks, whereas check plots had small, stunted plants that wilted in the heat of the day (Rhoades, 1979). No evidence of phytotoxicity was observed as a result of applying these nematicides. Norton and Hinz (1976) reported a 26% increase in corn yields after application of a carbofuran nematicide in sandy soils in Iowa. Apple (1971) similarly reported 6.9% maize yield increase after carbofuran application. According to Daynard *et al*. (1975), carbofuran is not inherently a stimulator of growth traits of corn but that it does control nematodes. Notably, some insecticides have been reported to have nematicidal properties hence indirectly additionally controlling parasitic nematodes in maize fields. For example, Rhoades (1979) reported that Terbufos, presently labelled only as a soil insecticide, provided good nematode control, and eventually resulted in higher yields when applied to maize. Effective chemical control of nematodes in maize could, however, be a useful production management tool when used in integrated nematode management systems (Johnson and Leonard, 1995). It is also recommended to inoculate soil planted to maize with effective nitrogen fixation agents, e.g., *Azospirillum* spp., to stimulate growth after treatment with nematicides (Fayez, 1990). As maize is a low value crop, it is important to take into consideration the environmental hazards and possible residual effects before any nematicides can be applied (McDonald and De Waele, 1987; Johnson and Leonard, 1995).

1.3.2 Cultural control

Cultural practices like crop rotation, tillage, planting time, application of organic amendments and sanitation are effective in reducing nematode populations (McDonald and Nicol, 2005).

In most crop rotations, however, maize has been erroneously used as a non-host of nematodes yet it is susceptible to various nematode species (Riekert and Henshaw, 1998). An effective crop rotation should target as many nematode species as possible (McSorley and Gallaher, 1992). Additionally, incorporating longer sequences of resistant crops before planting a susceptible crop is a highly effective crop rotation strategy (Johnson *et al.*, 1999). Knuth (2000) reported reduction of *Pratylenchus* spp. by radish as well as French and African marigold in maize-based rotations. However, rotation alone may not be sufficient to prevent subsequent susceptible crops from suffering nematode damage. Additional control strategies such as host plant resistance need to be integrated for effective management of plant-parasitic nematodes (Kinloch and Dunavin, 1993).

Tillage is important since several weed species have been reported to be hosts of a wide range of plant-parasitic nematodes (Salawu and Oyewo, 1999). Weeding of maize plots has been reported to reduce populations of *Ditylenchus* spp., *Heterodera* spp. and *Tylenchorhynchus clarus* (Youssef, 1998). It is highly recommended to combine tillage and rotation systems in nematode management strategies (Cabanillas *et al.*, 1999). Removal of old maize roots during weeding has also been reported as an important contribution to the cultural control of nematodes, which survive on these residues during the dry season (Egunjobi and Bolaji, 1979).

Fertilizer application in combination with early planting has been reported to reduce *Punctodera chalcoensis* nematodes in Mexico (Sosa-Moss, 1987). Ivezic *et al.* (1996) obtained up to 60% reduction in nematode populations dominated by *Pratylenchus thornei* in maize fields after application of potassium fertilizers. Large amounts of yard waste compost were reported to reduce *Paratrichodorus minor, Criconemella* spp., and *Pratylenchus* spp. in maize in the USA with a subsequent increase in yield (McSorley and Gallaher, 1996). However, the organic amendments had no effect on *Meloidogyne incognita* populations (McSorley and Gallaher, 1996).

1.3.3 Biological control

Parasitism and predation occur throughout nature. There is hence a natural biological control of nematodes wherever life processes are even and moderately sustained (Norton, 1978b). However, none of these biocontrol agents can be used economically in cereals at present (McDonald and Nicol, 2005). Some of the biological control agents that reduce nematode densities and damage are: viruses, bacteria, fungi, amoeba, sporozoan parasites, mites, predacious nematodes and insects (Norton, 1978b). On maize, only a few of these predators have been tested with most emphasis on fungi (Norton, 1978b).

Nematode-trapping fungi exist and have been effective in significant reductions of nematodes in maize. Bourne and Kerry (1999) reported significant reductions in *M. incognita*, *M. javanica* and *M. arenaria* in maize by the fungus *Pochonia chlamydosporia*. Bourne (2001) obtained 50% reductions in *M. incognita* after application of *Pochonia chlamydosporia* in rotations with maize and susceptible crops. More than 50% control of *Pratylenchus* spp. was achieved with application of *Paecilomyces lilacinus* (Gapasin, 1995). Other fungi such as *Glomus mosseae*, *Trichoderma pseudokoningii*, *Pseudomonas fluorescens* have been reported to improve maize grain yield by reducing *P. zeae* damage (Oyekanmi *et al.*, 2007). For example, field plots to which *G*. *mosseae* was applied with *P. zeae* had an increased root weight, leaf area and grain yield of 34.5%, 21.2% and 31.2%, respectively compared to plots with *P*. *zeae* only. Mycorrhizal fungi of the genus *Glomus* was reported to reduce *Meloidogyne chitwoodi* juveniles on maize (Estanol-Botello *et al.*, 1999). Strains of *Pseudomonas* spp. bacteria have been reported to inhibit invasion by *Meloidogyne* spp. and *Radopholus similis* in maize, tomato and banana roots (Aalten *et al.*, 1998).

1.3.4 Host resistance

Host resistance, which is the focus of this study, will be examined closely henceforth. It is the best management option as it is cheap and offers no technical difficulties to the farmer. In maize, pedigree breeding without selecting for nematode resistance may result in highly susceptible and intolerant crops, which could be very costly in any kind of production system (McDonald and Nicol, 2005). However, when intending to select for resistance to nematodes in maize, it is important to ensure that it is not at the expense of other commercially desirable traits (Jordaan and De Waele, 1987; Williams *et al.*, 1990).

1.4 Mechanisms of resistance to nematodes

Mode of nematode resistance in plants is influenced by the type of nematode and the plant species. Most plants will let infective stages of nematodes enter their roots freely, regardless of whether such plants are resistant or susceptible to nematodes (Katan, 1980; Egunjobi and Olaitan, 1986). Thereafter, infected cells of the plant will wither and die quickly (hypersensitivity) if the cultivar is resistant, thus blocking parasite development. Likewise, rates of growth of nematodes in incompatible host-parasite combinations are slower than in compatible combinations. In susceptible plants, a *Meloidogyne* female may begin egg production in three weeks but the life cycle of the same species in a resistant host under similar conditions may be extended to six weeks (Dropkin, 1989). Resistant plants also have a tendency to inhibit the growth of sedentary females, especially *Meloidogyne* spp. and

Heterodera spp., hence the sex ratio changes in favour of males, which require less nutrition than females. In addition, female fecundity is reduced in resistant cultivars, consequently suppressing nematode population development in these cultivars (Dropkin, 1989).

Pre-formed toxic (nematicidal) molecules in plants offer resistance against nematodes but require further investigation into their mode of action (Kahn, 1982). For example, terthienyl and bithienyl, which are effective against *Pratylenchus penetrans* and *Meloidogyne* spp., activate oxygen radicals, which in turn activate enzymes and damage cell membranes of nematodes (Dropkin, 1989). Some plants in the Cruciferae family contain isothiocyanates that inhibit hatching of juveniles from eggs of potato cyst nematodes. Leachates from *Asparagus officinalis* suppress populations of *Paratrichodorus,* probably by inhibiting cholinesterase, an enzyme of nerves and sense organs (Kahn, 1982). In fact, high levels of phenolic compounds have been recovered in tomato and tobacco cultivars resistant to *Meloidogyne* spp., compared to susceptible cultivars.

Mechanisms of resistance against nematodes have also been demonstrated in Lima beans (*Phaseolus lunatus*) and soybean against *Pratylenchus scribneri* and *Meloidogyne incognita*, respectively. In Lima beans, resistance to *P. scribneri* is as a result of accumulation of high concentrations of coumestrol and psoralidin compounds at sites of necrosis (Dropkin, 1989). In soybeans, cultivars resistant to *M. incognita* accumulate glyceollin in the root stellar tissues at concentrations well above the level that immobilizes the nematodes (van Gundy and McElroy, 1969). Although considerable progress has been made in examining modes of resistance to nematodes in crops, followed by breeding for resistant cultivars, such information is severely lacking for maize. A strategy to develop maize lines with acceptable levels of resistance to nematodes will require a clear understanding of the nature of inheritance of the resistance genes and the establishment of an appropriate screening method for nematode resistance in maize.

1.5 Resistance to nematodes in maize

Cultivars resistant to maize nematodes have been identified, developed and used across the globe. Most of the maize breeding work, screenings and selections have, however, been undertaken against the root-knot nematodes. This is because it is easier to identify resistance to sedentary than migratory endoparasites (Jordaan and De Waele, 1987). However, Johnson (1975) reported maize cultivars resistant to *Meloidogyne*, *Helicotylenchus* and *Paratrichodorus* species.

1.5.1 Resistance to *Meloidogyne* spp.

As regards root-knot nematodes, resistance has been reported to *M. incognita* and *M. javanica* (Ribeiro *et al.*, 2002), and *M. arenaria* (Sasser and Kirby, 1979). Windham and Williams (1994b) observed retarded growth or failure of juveniles to reach maturity in maize hybrids exhibiting resistance. A greenhouse screening of twenty-five commercial tropical hybrids recorded more susceptibility to *M. arenaria* than *M. incognita* (Windham and Williams, 1994a). However, according to Davis and Timper (2000), maize is generally more resistant to *M. arenaria* than *M. incognita*. Lordello and Lordello (1992) attributed resistance to *M. javanica* in maize to immunity existing in a parental line IAC Ip365-4-1 as a dominant trait. Diallel crosses between maize inbred lines demonstrated the importance of both general combining ability (GCA) and specific combining ability (SCA) affecting resistance to *M. javanica*, with Mp307 being the best source of resistance (Poerba *et al.*, 1990). Similarly, Mp307 offered better resistance to *M. incognita*, with GCA emerging as a significantly better source of variation than SCA (Williams and Windham, 1992). In the USA, open pollinated varieties (OPVs) have been screened as possible sources of resistance to *M. incognita*, with cv. Old Racoon and Tebeau offering more resistance than the resistant check (Aung *et al.*, 1990).

1.5.2 Resistance to *Pratylenchus* spp.

Resistance to several *Pratylenchus* species has been found in maize and its wild relatives (teosintes). Norton *et al.* (1985) reported resistance to *P. scribneri* in the wild maize species *Zea diploperennis* and *Z. mexicana.* The diploid *Z. diploperennis* crosses readily with maize, and fertile hybrids have been obtained by many breeders (De Waele and Elsen, 2002). Wicks *et al.* (1990a) developed and registered a yellow maize line with resistance to *P. hexincisus* and *P. scribneri* as well as to fungal diseases of ear rots and turcicum leaf blight. The line also has good SCA. Thomas (1980) found that commercial hybrids differed in their susceptibility to lesion nematodes, and that the degree of root colonization was relatively consistent across seven different soils. Improved maize hybrids were registered in the USA with resistance to *P. hexincisus*, namely SD101, SD102 and SD103 (Wicks *et al.*, 1990a; Wicks *et al.*, 1990b). The SD101 hybrid also offered resistance to *P. scribneri* (Wicks *et al.*, 1990a).

Several maize genotypes resistant to *P. zeae* and *P. brachyurus* have been identified (Lordello *et al.*, 1985). In the USA, maize cultivars Nab Elgamal, Early American and Giza Baladi exhibited less damage from *P. zeae* than did cultivars Single Cross 14 and Double Cross 67 (Fortuner, 1976). In Kenya, hybrids H627 and Pan5195 have been reported to suppress *P. zeae* reproduction, whereas H624, H625, H511, H512, Dryland composite 1,

Katumani composite and Pwani hybrid are susceptible to *P. zeae* (Kimenju *et al.*, 1998; Arim *et al.*, 2006). In Nigeria, inbreds 9450 and 5057, and an OPV called Western Yellow were described as resistant to *P. zeae* (Oyekanmi *et al.*, 2007). On the other hand, OPVs Gandajika 8022 and Rilemne 88 TZSR-Y-1 were confirmed susceptible to *P. zeae* (Oyekanmi *et al.*, 2007). Using segregating populations obtained from the lines Col 2(22) (resistant) and Ip48-5-3 (susceptible), Sawazaki *et al*. (1987) concluded that resistance to *P. zeae* and *P. brachyurus* in maize was due to two dominant genes with an additive effect.

1.5.3 Resistance to other maize nematodes

High levels of resistance in commercial hybrids have been reported to the semi-endoparasitic nematode *Rotylenchulus reniformis* based on reproduction of the nematode (Windham and Lawrence, 1992). Hashmi *et al*. (1993) reported resistance to *Heterodera zeae* in maize inbred lines observed through greenhouse screening experiments. Singh and Patel (1999) reported a maize variety resistant to *Tylenchorhynchus vulgaris*, and Venditti and Noel (1995) reported a variable genotype reaction to *T. zambiensis* in maize. Norton *et al.* (1985) reported resistance to *Helicotylenchus pseudorobustus* in the wild maize species *Z. diploperennis* and *Z. mexicana.*

1.6 Rearing *Meloidogyne* and *Pratylenchus* spp.

For controlled nematode screening trials, adequate inoculum preparation of the intended nematode species is a prerequisite. Inoculum preparation may be simple and cheap for some nematode species but cumbersome for others. Stock cultures of *Meloidogyne* spp. are routinely maintained in pots on very susceptible hosts, such as tomato (*Lycopersicon esculentum* Mill.) or aubergine in a greenhouse (Hussey and Barker, 1973; Hussey and Janssen, 2000). Second stage juveniles, egg masses or egg suspensions from galled tomato roots can be used as inoculum (Hussey and Barker, 1973).

The sterile carrot disc technique has successfully been employed for the monoxenic culture of a number of root-lesion nematodes, such as *P. vulnus* (Moody *et al.*, 1973), *P. brachyurus* (O'Bannon and Taylor, 1968), *P. goodeyi* (Pinochet *et al.*, 1995), *P. sudanensis* (Mudiope *et al.*, 2004), and *P. scribneri* (Lawn and Noel, 1986). Excised maize roots have been recommended for the monoxenic culture of *P. penetrans* (Tiner, 1960) and *P. zeae* (Meyer, 1984; Jordaan and De Waele, 1988; Bridge, 1994). For *P. zeae*, the method is laborious in terms of media preparation and ensuring nematode penetration into the excised maize roots from the smooth agar medium (Meyer, 1984). It is also expensive if Murashige and skoog medium is used as described by Kimenju *et al.* (1998).

Alfalfa callus tissue has also been used to culture monoxenically populations of *P. brachyurus, P. coffeae, P. crenatus* and *P. zeae* (Motalaote *et al.*, 1987). The method is also laborious and results in low nematode populations unless the nematode populations generated are further reared in sandy loam soils before use as inoculum (Martin *et al.*, 1983).

Sterile carrot discs offer a cost effective and relatively less laborious alternative for rearing nematodes, which can result in greater nematode multiplication compared with other methods (Speijer and De Waele, 1997). However, not all migratory plant-parasitic nematodes are suitable for rearing on carrot discs. For example, attempts to raise *Helicotylenchus multicinctus* were reported as unsuccessful (Speijer and De Waele, 1997). The efficiency of sterile carrot discs for mass culturing of *P. zeae* is not known. Therefore, the current study focused on assessment of the possibility of raising *P. zeae* inoculum on carrot discs.

1.7 Recurrent selection

Recurrent selection includes selection methods that are conducted in a repetitive manner (cyclical scheme) in which the best individuals resulting from a first selection cycle are crossed to generate the material for the next selection cycle regardless of the method used to manage the progeny of these crosses (Hallauer and Miranda, 1988; Sprague and Dudley, 1988; Rubaihayo, 1996). Recurrent selection is the cornerstone for population improvement in cross-pollinated crops. Selection is usually conducted for quantitative traits in genetically broad based populations (Sprague and Dudley, 1988). These populations can then serve as a potential source of superior inbreds and can hinder development of a possible genetic ceiling for future hybrid improvement (Duvick, 1992; Kannenberg and Falk, 1995). Regardless of the trait under selection, the objectives of recurrent selection are to: i) increase the frequency of favourable alleles needed to change the mean of the population in a favourable direction; and ii) maintain genetic variability for continued selection by inter-mating superior progeny in each cycle of selection (Rubaihayo, 1996).

Recurrent selection methods can be categorized as inter- or intra-population selection. Inter-population recurrent selection (reciprocal recurrent selection) is a cyclical breeding procedure in which progressively improved populations of two germplasm pools are used reciprocally as testers and direct effects of selection are estimated in the population cross (Doerksen *et al.*, 2003). Intra-population selection involves the improvement of a single population. It is particularly effective for improving pest resistance, adapting germplasm for specific environments, and changing the genetic composition of the grain (Hallauer and

Miranda, 1988). Compared to inter-population selection, intra-population selections are more commonly used because of simplicity, and their applicability for a greater number of traits. The most common intra-population methods employed in recurrent selection are paternal or maternal half-sib families; full-sib families; S_1 and S_2 inbred lines (Ramírez-Díaz *et al.*, 2000).

Population improvement through selfed progeny selection (S) is the result of direct selection favouring additive genetic effects because there is no masking effect of a tester (Hallauer and Miranda, 1988). In the S scheme, the selection units are either S_1 or S_2 family means compared with the grand mean of all the S_1 or the S_2 families, respectively. Remnant seeds from the selfed ears are used for recombination (Hallauer and Miranda, 1988). Inbred progeny selections are amenable to applied breeding programs and can be easily integrated with the other aspects of pedigree selection. Inbred progeny selection is also effective for exposing deleterious recessive alleles and, consequently, reducing the genetic load from the populations under selection (Doerksen *et al.*, 2003).

The S_1 progeny recurrent selection scheme has been used in performance improvement, in particular, for various crops (Hallauer and Miranda, 1988). In the current study, selection was based on S_1 progeny and not half-sib or full-sib families because quantitative genetic studies indicated that additive genetic effects with partial to complete dominance were of greater importance than non-additive genetic effects in maize populations (Sprague and Dudley, 1988). Hence selection methods that emphasize selection for additive effects are more appropriate and effective in the improvement of most maize traits (Hallauer and Miranda, 1988). The coefficients for the additive genetic component of variance among progeny are 1.0 for S_1 progeny, 1.5 for S_2 progeny, and nearly 2 for S_n progeny, but only 0.25 among half-sib and 0.5 among full-sib families. Therefore, the expected genetic variation, assuming only additive genetic effects, among S_1 progeny is expected to be four times greater than among half-sib families and twice that among full-sib families (Hallauer and Miranda, 1988). These expected differences for efficiency of selection are also reflected in the empirical estimates of heritability. As a result, selection among S_1 or S_2 families is useful for characters having low heritability, because a larger portion of additive genetic variance contributes to genetic advance than with full-sib or half-sib selection.

Some studies have been conducted on improving nematode resistance using recurrent selection breeding techniques. For example, through recurrent selection and progeny testing, five bitter almond (*Prunus amygdalus*) genotypes indicated dominance of resistance to *Meloidogyne javanica* (Kochba and Spiegel-Roy, 1975). After seven cycles of recurrent selection, partial resistance was achieved against *M. trifoliophila* in white clover (*Trifolium*

repens) (Mercer *et al.*, 2000). Five cycles of phenotypic recurrent selection for early vigour in red clover (*Trifolium pratense*) decreased root gall scores by 0.20, 0.33, and 0.26 units per cycle and decreased egg mass scores by 0.42, 0.32, and 0.30 units per cycle (1–5 rating scale) when the cycles were infested with *M. arenaria*, *M. incognita*, and *M. javanica*, respectively (Quesenberry *et al.*, 1989). Two additional cycles of greenhouse half-sib family selection lowered gall scores by 0.45 and 0.31 units per cycle in the *M. arenaria* and *M. javanica* populations, respectively, but only 0.16 in the *M. incognita* population (Quesenberry *et al.*, 1989). In S_1 evaluations of pearl millet, each of the four cultivars was heterogeneous for resistance to *M. incognita* but the progeny varied from highly resistant to highly susceptible (Timper and Wilson, 2006). The S_1 recurrent selection improves upon a trait by exploiting the additive genetic effects, therefore a suitable approach for maize population improvement. The current study serves as a baseline for determining the effectiveness of recurrent selection to improve nematode resistance in maize.

1.8 Participatory rural appraisal

Breeders are often not well informed of the special needs and preferences of farmers (Toomey, 1999; Bänziger and de Meyer, 2002). The formal breeding sector has therefore often encountered setbacks in developing new cultivars. First, most new cultivars have been unacceptable to farmers (Witcombe *et al.*, 1996), and secondly, breeders have discarded many crosses because of traits considered undesirable, but which may actually be of interest to farmers (Toomey, 1999; Bänziger and de Meyer, 2002). This scenario has resulted because in formal plant breeding, the relative importance of various characters can be described by a selection index which does not correspond to farmers' preferences. Farmers are not necessarily interested in yield *per se,* but also other traits and combinations thereof. For example, Gibson *et al*. (2005) listed cob size, kernel size, and colour as farmers' major preferences in maize in Uganda. According to Sthapit *et al*. (1996), farmers' maize trait priorities range from grain colour, yield, plant height and maturity period. Participatory rural appraisal (PRA) is therefore an ideal tool to link the needs of rural communities to the activities of breeders, and to enable them do their own investigations, analysis, presentation, and planning of issues affecting them (Chambers, 1994). Participatory rural appraisal also has the advantage of generating and articulating knowledge in more participatory ways, and sharing of this knowledge in a non-threatening manner. This is undertaken through modelling, diagramming, ranking, and quantification procedures, cross checking (triangulation), among other methods (Chambers, 1994).

The involvement of farmers in breeding or setting the breeding objectives is not a novel concept in Africa. Witcombe *et al.* (1996) reported that involvement of farmers in breeding

helped breeders to appreciate the unique requirements and preferences for farmers resulting in the selection of appropriate germplasm. Bett *et al.* (2000) reported that farmers in Kenya were interested in selecting maize varieties for tolerance to drought and low soil nitrogen stress. In Ghana, a farmer participatory varietal selection (PVS) programme led to the identification and spread of upland rice varieties, which met the combined requirements of farmers, traders and consumers (Dorward *et al.*, 2007). Little effort has been devoted to establishing farmers' perceptions of nematodes and the damage they cause on numerous crops, in Africa, including maize.

1.9 Conclusions from review of literature

From the literature reviewed, nematodes are an underestimated problem across Africa, which undermine crop production, especially in resource poor conditions, leading to inefficiency of water and nutrient use. Development of elite maize germplasm with resistance against the plant-parasitic nematodes, *Pratylenchus zeae* and *Meloidogyne* spp. in particular, without compromising yield potential, would appear a highly relevant and beneficial breeding objective for this important staple crop in Africa. Breeding for resistance is appropriate given the poor perception of nematode problems, the availability of resistance genes and the potential economic returns over time. There is, however, need to understand cheaper and reliable methods of raising nematode inoculum to be used in the evaluation trials. The current study would thus test the feasibility of using carrot discs to rear *P. zeae*. *Meloidogyne* spp. will be maintained on tomatoes growing in pots.

Nature of gene action and responses to selection for nematode resistance in maize will be investigated by conducting a diallel analysis and through an S_1 progeny recurrent selection study, respectively. Diallel analysis is preferred because it is possible to make any cross among a collection of genetic entities, and permits the estimation of additive, dominance and environmental effects, on addition to recognizing non-allelic interactions. Preference for S_1 progeny selection to sib selection is that in S_1 progeny selection, a larger portion of additive genetic variance contributes to genetic advance than in sib selection. A PRA would help to ascertain farmers' perceptions of nematodes, their understanding of nematode damage and maize varietal preferences in Eastern and southern Uganda. The farmers' views would be considered during selection to enable development of nematode resistant cultivars which are preferred by farmers.

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CHAPTER TWO

[2]Awareness of plant-parasitic nematodes and preferred maize cultivars among smallholder farmers in East and Southern Uganda: implications for assessing nematode resistance breeding needs in African maize

Abstract

In Uganda, nematodes have the potential to cause substantial yield losses, yet it is not known whether farmers have knowledge of the damage these pests cause. A participatory rural appraisal was therefore conducted to assess farmers' awareness of nematodes, and to determine the preferred traits in new maize germplasm. Data were collected from 120 households in two maize-growing districts. Maize roots and soil samples were also collected from farmers' fields, and nematode incidence determined. A small percentage (18.5%) of farmers was familiar with nematodes and the related damage on maize. *Pratylenchus zeae* occurred at generally higher frequencies than *Meloidogyne* spp. in the susceptible cultivars. The landraces and the cultivar Longe 5, which reportedly gives the lowest yields, supported high nematode populations. Farmers' most preferred traits were pest and disease resistance, high grain palatability, long storage duration and large kernels. These traits need to be integrated in a breeding programme for nematode resistance in maize.

Keywords: Maize, *Meloidogyne* spp., Nematodes, Participatory rural appraisal, *Pratylenchus zeae*.

2.1 Introduction

Maize (*Zea mays* L.) contributes 15-50% of the energy in human diets in sub-Saharan Africa (Bänziger and Diallo 2002). In Uganda, annual consumption is around 23 kg per capita (NARO 2002). Productivity, which approximates 1.5 t ha^{-1} is, however, low compared to an average of 3 t ha^{-1} (FAOSTAT, 2007) achieved in other developing regions where irrigation, fertilizer and pesticides are widely used to maximise yield.

Potential grain yield losses have been reported in maize as a result of nematode infestation. In Kenya, Bridge (1994) estimated 50% yield loss due to root-lesion nematodes. A 12% yield loss was reported in South Africa due to *Meloidogyne* spp. (Keetch 1989). In Uganda, no germplasm is known to be resistant to nematodes. Hybrids such as Longe 7H and DK 8031, which are being advocated for use to improve maize productivity, are vulnerable to attack by nematodes, which are widespread in most maize-growing districts of Uganda (Butseya *et al.,*

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2005). Potential methods of management in sub-Saharan conditions are often not practicable at a field scale, e.g. the application of fumigants and solarisation. Breeding nematode-resistant maize cultivars provides the greatest potential economic dividend for long-term management of nematodes, particularly given the environmental and health concerns associated with the use of nematicides (Starr *et al.*, 2002).

In order to enhance adoption of new varieties, farmers need to be engaged early in the varietal development process (Witcombe *et al.,* 1996). Farmers usually consider the total package of the varieties as reflected by overall grain yield, grain quality and end-user traits. For a breeder working in isolation, these may be difficult to quantify to the satisfaction of farmers. The involvement of such farmers in breeding or setting the breeding objectives offers the best option of ensuring a product with high potential adoption. Witcombe *et al.* (1996) reported that involvement of farmers in breeding helped breeders to appreciate the unique requirements and preferences of farmers, and resulted in the selection of appropriate germplasm. The aims of this study were to: (i) assess farmers' perceptions on nematodes; (ii) establish the nematode infestation levels on maize in farmers' fields; and (iii) determine other important traits that farmers value in a maize cultivar.

2.2 Materials and methods

2.2.1 Selection of sites and farmers

A participatory rural appraisal (PRA) was conducted from November 2007 to January 2008 in the tropical-maize growing districts of Iganga (00^o 37.347' N; 033^o 381' E; 1157 m.a.s.l) and Masaka (00^o 21.194' S; 031^o 33.290' E; 1218 m.a.s.l) located in eastern and southern Uganda, respectively. These districts receive mean annual rainfall in excess of 1 000 mm, which is adequate for maize production. Farmers from parishes (sub-division within a sub-county comprising at least two villages) with predominantly sandy-loam soils were targeted. Sandy soils are generally associated with a higher incidence of nematodes than heavier soils (Norton, 1978). Two sub-counties (sub-division within a county comprising at least two parishes; a minimum of two counties form a district) were selected per district, with 30 farmers each to participate in the PRA, providing a total of 60 participating farmers per district and 120 farmers in total. The local extension staff database of maize farmers were used as the sampling frame. Random sampling was used resulting in almost equal numbers of female and male respondents.

2.2.2 Data collection

Data were collected using focus group discussions, semi-structured interviews and transect walks. The focus group discussions were conducted with 10 farmers comprising maize-

growers and opinion leaders with the aim of identifying the issues of concern in each district. Semi-structured interviews were conducted, based on the outcome of the focus group discussions, with 15 farmers per parish. Using an interview guide, questions were introduced to the group, with key informants and translators clarifying issues where necessary and enumerators recording the information. The major elements during interviews were: (i) maize production constraints and problems of nematodes; (ii) maize cultivars grown; (iii) estimated yield; and (iv) preferred traits in new maize cultivars. Identification of nematode damage symptoms was aided by displaying photographs showing infected plants and roots of maize, tomatoes (*Lycopersicum esculentum*), cassava (*Manihot esculenta*) and bananas (*Musa* spp.). Matrix ranking, to identify farmers' priorities, was conducted by dividing the 15 farmers randomly into three groups of five each. Elements for ranking were performance of cultivars, preferred traits, production constraints, and the pest and disease control practices. The elements were placed along the horizontal axis of the matrix, and the criteria for evaluating each item were placed along the vertical axis of the matrix. The farmers then ranked each element using a scale of 1 (most important) to 5 or 6 (least important). Transect walks were made with key informants and farmers through fields and village living-areas mainly to: (i) observe the general land use; (ii) identify crops cultivated; (iii) collect soil and maize root samples; and (iv) to collect seeds of the local landraces for incorporation in the breeding programme. The maize farmers visited were selected randomly from the maize farmers' database obtained from the extension staff. Transect walks were done with key informants starting from nearby fields and ending with fields furthest from the sub-county headquarters.

Roots with the soil embedded were picked from at least five sampling points for each maize field visited. The soil and chopped roots were then separately bulked into a composite sample, before sub-sampling. Nematode damage indices of root-knot galls and blackened root lesions were visually counted from 20 g root fresh mass per sample. Next, nematodes were extracted from 5 g of macerated fresh roots and 100 g soil sub-sample, using a modified Baermann sieve method (Coyne *et al.,* 2007) at the IITA laboratory in Namulonge, Uganda (Appendix 2.1). All samples were examined after a 48 h extraction period and nematodes counted using a Leica Wild M3C stereomicroscope. Nematode populations in the soil were estimated using the whole suspension with the help of a 20 µm sieve, whereas the nematode populations in the roots were estimated from triplicate 2 ml aliquots drawn from a 25 ml suspension.

2.2.3 Data analysis

Qualitative data were coded and analysed using SPSS version 15.0 (SPSS, 1989-2006). Chi-squared tests were used to compare performance between locations and Kendal's tau-b

correlations run for non-parametric data. Analysis of variance (ANOVA) and paired t-tests, using Proc ttest procedure in SAS version 9.1 (SAS, 2002-2003), were applied to quantitative data. F-values and t-values of nematode counts were based on square root (sqrt[x+1])-transformed data, with pooled variance (P) used for equal variance and Satterthwaite approximation (S) used for unequal variance.

2.3 Results

2.3.1 Farmers' perceptions of nematodes

Only 18.5% of respondents recognised symptoms of nematode damage in maize in both locations (Table 2.1). However, many farmers (70%) were familiar with the symptoms of nematode attack on bananas, cassava and tomatoes but did not necessarily associate them with nematodes. Farmers were more familiar with nematode symptoms in Masaka than Iganga. Only 15.8% of the farmers could associate yield losses with nematode infection, most of these had planted maize in rotation with tomatoes, eggplants (both the white and purple cultivars of *Solanum melongena*) or bananas.

Table 2.1: Incidence of plant-parasitic nematodes and farmers' perception of nematodes in two districts of Uganda

District (n = 120)	Familiarity with nematode damage on crops	Incidence of Nematodes on maize	Frequency of farmers who associated nematodes with yield reduction
	†Respondents (%)		
Iganga (n=60)	53.3	16.9	18.3
Masaka (n=60)	86.7	20.0	13.3
Average (n=120)	70.0	18.5	15.8
Chi-squared	15.9	0.184	0.563
Prob. (df =1)	0.0	0.668	0.453

†Frequencies of respondents who answered 'No' have been omitted, though are catered for in the chi-squared value

2.3.2 Nematode related symptoms observed by farmers

The major nematode related symptoms observed on maize included leaf chlorosis, stunted plants and patchy growth (Fig. 2.1). Root-knot galling and shoot weight reduction were the least observed symptoms. However, very few farmers were aware that the observed symptoms could be a result of nematode infection.

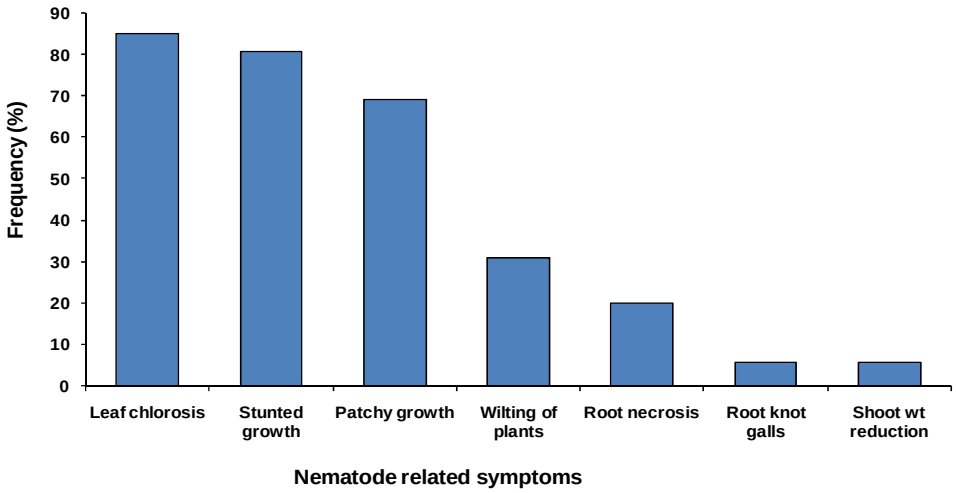

Figure 2.1: Nematode-related symptoms observed on maize in farmers' fields in Iganga and Masaka districts in Uganda during 2007-2008.

Using Kendall's tau-b non-parametric correlation method, significant positive correlations ($P \leq 0.05$) were obtained between leaf chlorosis and stunted growth, leaf chlorosis and patchy growth, and between stunted growth and patchy growth (Table 2.2). Root-knot galling only had a positive correlation with nematode damage symptoms of necrotic root lesions and plant wilting. Necrotic root lesions were positively ($P < 0.001$) correlated with plant wilting, but wilting was not associated ($P > 0.05$) with patchy growth or shoot weight.

Table 2.2: Kendall's tau-b correlation coefficients between nematode-related symptoms observed by farmers on maize in Uganda

Symptoms	Leaf chlorosis	Root-knot galls	Stunted growth	Root necrosis	Wilting of plants	Patchy growth
Root-knot galls	-0.095ns					
Stunted growth	0.329***	-0.059ns				
Root necrosis	-0.257**	0.053ns	-0.233**			
Wilting of plants	-0.427***	0.065ns	-0.271**	0.388***		
Patchy growth	0.275**	-0.219*	0.179*	-0.568***	-0.609***	
Shoot weight reduction	-0.095ns	-0.062ns	0.031ns	0.053ns	-0.089ns	0.012ns

***, **, * Correlation coefficient is significant at $P \leq 0.001$, $P \leq 0.01$ and $P \leq 0.05$, respectively;
ns, correlation coefficient is non-significant ($P > 0.05$).

2.3.3 Nematode populations and related symptoms

Pratylenchus zeae and *Meloidogyne* spp. were the most frequently encountered nematodes both in maize roots and soil (Table 2.3). Other nematode species that were recovered, especially from soil samples, in relatively high densities were *Helicotylenchus* spp.,

Scutellonema spp., *Rotylenchus* spp., *Tylenchus* spp., *Aphelenchoides* spp., *Pratylenchus brachyurus* and *Ditylenchus* spp.

Table 2.3: Nematode densities, damage symptoms and standard errors on maize recorded from fields in Iganga and Masaka Districts, Uganda

		Roots (populations per 100 g root)				Soils (populations per 100 g of soil)	
District	n	*P. zeae*	*Meloidogyne* spp.	No. of galls	Root lesions no.	*P. zeae*	*Meloidogyne* spp.
Iganga	23	12656±10321	477.3±129	7.0±3.2	6.1±2.6	19.3±9.4	12.1±4.2
Masaka	17	526.0±235	432.3±117	3.8±2.0	3.8±2.7	0.88±0.4	6. 3±2.5
Mean		6590.8	454.8	5.4	5.0	10.1	9.2
F-value		40.76***	1.35ns	1.99ns	1.41ns	48.5***	2.05ns
t-value		1.98*^{S}	-0.07ns^{P}	0.55ns^{P}	0.66ns^{P}	1.97*^{S}	1.03ns^{S}

***, **, * Significant at $P \leq 0.001$, $P \leq 0.01$ and $P \leq 0.05$, respectively; ns, non-significant (P > 0.05); P = pooled variance, S = Satterthwaite approximation.

Numbers of *P. zeae* were significantly (P < 0.001) higher in Iganga than Masaka district. Root-knot galling and root lesions, which are associated with *Meloidogyne* spp. and *Pratylenchus* spp. infection respectively, were observed on some maize cultivars, but their levels did not significantly differ across districts.

Numbers of *Meloidogyne* spp. varied little among the commonly grown cultivars namely Longe 1, Longe 5 and the local landrace, ranging between 386.5 and 535.3 *Meloidogyne* spp. per 100 g root fresh mass, and between 7.2 and 9.6 *Meloidogyne* spp. per 100 g of soil (Table 2.4).

Table 2.4: Nematode densities, related damage and standard errors (per 100 g of root or soil) across cultivars sampled in farmers' fields in Iganga and Masaka

Cultivar Name†	n	*Meloidogyne* spp. (root)	*P. zeae* (root)	No. of galls	Root lesion no.	*Meloidogyne* spp. (soil)	*P. zeae* (soil)
Landrace[l]	13	535.3±185.3	21042±18215	1.54±0.8	9.2±4.3	9.6±4. 2	7.0±5.7
Longe 1	8	395.8±179.4	417±143	7.5±3.7	5.0±2.6	7.8±2 .7	3.6±3.0
Longe 5	14	386.5±144	1112±384	7.1±4.5	2.9±1.9	7.2±3 .5	22.2±14.5
Mean		439.2	7523.7	5.4	5.7	8.2	10.9

†Cultivars not shown were either not available in farmers' fields at the time of sampling or only few farmers had them; [l]The landraces were 'Mawalampa' in Iganga and 'Omunandi' in Masaka.

In both the root and soil samples, the local landrace had the highest incidence of *Meloidogyne* spp. whereas Longe 5 had the lowest. *Pratylenchus zeae* populations ranged

from 417 (Longe 1) to 2 1042 (Local Landrace) per 100 g root fresh mass and from 3.6 (Longe 1) to 22.2 (Longe 5) per 100 g of soil across cultivars. Nematode damage symptoms of root-knot galling and root lesions did not significantly vary across cultivars. Other cultivars such as DK8031, Zimbabwe and Longe 4, though present, were being cultivated by one to two farmers and were therefore not included in the mean comparisons. However, a substantial number of nematodes were observed on these cultivars.

2.3.4 Farmers' preferred traits

High grain yield was the most preferred trait in both districts (Table 2.5). This was followed by resistance to pests and diseases, palatability and grain storability. Lodging resistance and drought tolerance were the lowest ranked traits. Besides the aforementioned traits, farmers, especially in Masaka, stressed their preference for large kernels, which they associate with increased seed mass and higher market prices than small kernels. Large kernel preference and palatability were cited as the major reasons for continued cultivation of their local landraces as opposed to hybrids.

Table 2.5: Farmers' ranking of most desirable traits in a maize cultivar in Uganda

	Respondents (%)			
Trait	Iganga	Masaka	Mean	Rank
High yielding	42.6	55.0	49.1	1
Pest and disease resistant	25.9	6.7	15.8	2
High palatability	3.7	18.3	11.4	3
Early maturing	3.7	8.3	6.1	4
High storability	13.0	0.0	6.1	4
Large kernels	1.9	8.3	5.3	5
Lodging resistance	5.6	1.7	3.5	6
Drought tolerance	3.7	1.7	2.6	7

2.3.5 Estimated yield of the cultivars and associated production constraints

Grain yield was reported to be between 1 and 2 t ha^{-1} by most respondents (Table 2.6). Cultivars identified with the highest nematode populations, e.g. Longe 5 and the landrace (Table 2.4) also produced the lowest yields (<1 t ha^{-1}), and planting seed of these cultivars were reported to be the most frequently recycled as home-saved seed. Only a few farmers (0.8%) reported yields greater than 5 t ha^{-1}. These were recorded on cultivars Longe 1, Zimbabwe and DK 8031.

Table 2.6: Farmers' estimated yields (%) for the cultivars they grow in Iganga and Masaka in Uganda

Estimated on-farm yield (t ha^{-1})	Number of respondents for yield estimate (n)	Cultivar					
		Longe 1 (OPV)	Longe 2 (Hybrid)	Longe 5 (OPV)	Landrace (OPV)	Zimbabwe (Hybrid)	Mean
		Respondents (%)					
<1	13	7.4	0.0	15.2	8.8	11.1	8.5
1 - 2	65	37.0	75.0	43.5	70.6	22.2	49.7
2.1 - 3	26	29.6	0.0	21.7	14.7	33.3	19.9
3.1 - 5	25	22.2	25.0	19.6	5.9	11.1	16.8
5.1 - 7	2	0.0	0.0	0.0	0.0	11.1	2.2
> 7	2	3.7	0.0	0.0	0.0	11.1	3.0
Number of respondents per cultivar (n)		27	8	46	34	9	
SE (mean)		1.67	0.16	1.16	1.17	3.05	

[T]Cultivars Longe 4, DK 8031, PAN67 and Kenya hybrid not shown because <5 farmers were growing them with yields hardly exceeding 3 t ha^{-1}except for DK 8031.

Most farmers (58.3%) ranked pests and diseases as the major constraints to maize production (Table 2.7). They particularly reported stem-borers and *Striga* spp. as the leading pests in Masaka and Iganga, respectively.

Table 2.7: Production constraint ranking in importance across districts (%) in Uganda

Constraint	Rank	District		
		Iganga (%)	Masaka (%)	Mean across districts (%)
Pests and diseases	1	48.3	68.3	58.3
Drought	2	15.0	13.3	14.2
Low soil fertility	3	16.7	8.3	12.5
Lack of improved cultivars	4	13.3	5.0	9.2
Expensive labour	5	0.0	5.0	2.5
Inadequate marketing channels	6	3.3	0.0	1.7
Other	7	3.3	-	1.7
Floods	-	-	-	-

- indicates a practice not ranked

The most rampant diseases were turcicum leaf blight (TLB), caused by *Exserohilum turcicum* and maize streak virus (MSV) transmitted by leafhoppers of the genus *Cicadulina*. Other highly ranked production constraints in both districts were drought, low soil fertility, and lack of improved cultivars. Farmers also mentioned inadequate marketing channels and natural flooding as major obstacles to maize production in Iganga and Masaka, respectively.

2.4 Discussion

Results from the present study, and those of Butseya *et al.* (2005), show that the nematode problem is widespread on maize in Uganda. The positive correlation among nematode symptoms of patchy growth and chlorosis is an indication of nematode damage. Patel *et al.* (2002) reported considerable reduction in root and shoot weight, plant height and chlorophyll content and an almost ten-fold increase in *P. zeae* numbers in maize infected with *P. zeae* compared to nematode-free maize.

The high populations of plant-parasitic nematodes in the root and soil samples obtained from farmers' fields suggest that nematodes contribute to maize yield losses in the surveyed districts. Predominantly sandy-loam soils have been reported to favour survival of *P. zeae* and *Meloidogyne* spp. in maize fields (Norton, 1978). This was the case particularly in Iganga district. Similarly, the cultivars grown in these districts lack genetic variation for nematode resistance since they were all susceptible.

Farmers' ranking of pest resistance as crucial in the breeding programme could have been prompted by their experience with stem-borers and *Striga* spp., whose damage is easily noticeable, unlike that of nematodes. The major reasons for continued cultivation of landraces as opposed to hybrids were large kernel size and palatability associated with landraces. Previous researchers reported similar findings with respect to kernel characteristics preferred by farmers in east and southern Africa (Gibson *et al.,* 2005). These traits need to be selected for in any maize breeding programme in Uganda.

The grain yield of 1-2 t ha^{-1} was similarly reported by NARO (2002) among smallholder farmers in Uganda. The cultivars identified with such low yields such as Longe 5 and the landrace also had supported high nematode populations, among other stresses. Farmers' over-dependence on home-saved seed as their planting material could have also contributed to the low yields. According to Gibson *et al.* (2005), farmers buy seed but grow them for many cropping cycles leading to a breakdown in resistance to various stresses as a result of out-crossing with susceptible varieties planted in neighbouring fields.

Turcicum leaf blight (TLB) and maize streak virus (MSV), which were the most rampant diseases, are not new to Uganda (Bigirwa *et al.,* 1993). The other highly ranked production constraints in both districts were drought, low soil fertility, and lack of improved cultivars. Plant-parasitic nematodes have particularly been reported to be aggravated by drought and other stresses such as low soil fertility and diseases (Swarup and Sossa-Moss, 1990). Breeding interventions are the most appropriate means of alleviating the stresses

encountered in this study. The other obstacles to maize production, such as inadequate marketing channels and natural flooding, require intervention by other partners in rural development.

2.5 Conclusion

This study demonstrated that nematodes are present on most cultivated maize varieties in farmers' fields in Uganda. *Pratylenchus zeae* was particularly prominent and was responsible for most of the damage. However, very few farmers are aware that nematodes are a serious maize production constraint. Since most varieties cultivated by farmers in Iganga and Masaka districts of Uganda were nematode-susceptible, the best management approach would be breeding cultivars with nematode resistance. The breeding aspect would be tackled by either sourcing exotic resistant germplasm for introgression into locally adapted germplasm or using a population improvement approach on selected cultivars. Farmer-participatory approaches at the beginning of a breeding project would enable breeders improve the selection procedure by taking into account farmers' preferred traits at an early stage.

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Appendices

Appendix 2.1: Site description and location of IITA-Namulonge farm

The International Institute of Tropical Agriculture (IITA) farm is located at Namulonge village, Busukuma subcounty, Wakiso district. The area is sometimes referred to as IITA – Sendusu, a name it acquired from the original owner of the farm. Namulonge farm is in the mid elevation humid forest zone, 28 km north of Kampala (32°34'E, 0°32'N) at 1200 m a.s.l. It has an average rainfall of approximately 1300 mm, an average annual temperature of 22℃.. The soils are dark reddish-brown sandy loam orthic ferralsols with a pH range of 5.5 to 6.2 (IITA, 1992).

Location of IITA-Namulonge farm

Figures are modifications of maps obtained from http://en.wikipedia.org/wiki/Namulonge (A) and http://maps.google.co.ug/maps?hl=en&tab=wl&q=namulonge (B)

CHAPTER THREE

[3]Monoxenic culture of *Pratylenchus zeae* on carrot discs

Abstract

Pratylenchus zeae is widespread on maize in Uganda but studies to aid in screening cultivars for resistance to this nematode have been constrained by lack of a cheap and reliable technique for raising inoculum. Use of excised maize roots to culture *P. zeae* is laborious in terms of media preparation and ensuring nematode penetration of roots. Sterile carrot discs are more cost effective and relatively less laborious for rearing most root-lesion nematodes but information on their effectiveness for *P. zeae* is lacking. The objective of this study was to assess the efficiency of sterile carrot discs for mass culturing of *P. zeae* collected from maize roots. Twenty live nematodes were transferred to the margins of each of 40 sterile carrot discs contained in 3.5 cm diameter sterile glass Petri dishes. All cultures were maintained in the dark at 25 ± 1℃. The reprod uction of the nematodes was assessed three months after inoculation, when the nematodes accumulated on the surface of most carrot discs and the Petri dish surface. The study revealed a mean density of 63 913 vermiform *P. zeae* comprising 46% females, 54% juveniles and no males. Overall reproduction rate of *P. zeae* was 5 090 times, whereas the females alone increased by a factor of 1 476. Therefore, use of carrot discs to culture *P. zeae* results in higher reproduction rates compared to excised maize roots.

Key words: Mass rearing, Reproduction, Root-lesion nematodes.

3.1 Introduction

Pratylenchus zeae Graham is a key nematode pest of a number of tropical and sub-tropical graminaceous crops (McDonald and Nicol, 2005). In Uganda, *P. zeae* is the most important nematode pest of maize (Butseya *et al.*, 2005) though detailed studies of this nematode on maize remain limited. Inoculation studies will help provide key information on the pest potential of *P. zeae* and in developing resistant cultivars through a screening facility. A successful mass culturing method for *P. zeae* would, therefore, be useful for such procedures.

The sterile carrot disc technique has successfully been employed for the monoxenic culture of a number of root-lesion nematodes, such as *P. vulnus* Allen *et* Jensen (Moody *et al.*, 1973), *P. brachyurus* (Godfrey) Filipjev *et* Shuurmans Stekhoven (O'Bannon and Taylor,

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1968), *P. sudanensis* Loof *et* Yassin (Mudiope *et al.*, 2004), and *P. scribneri* Steiner (Lawn and Noel, 1986). This method has not been reported for rearing *P. zeae*. Excised maize roots have been recommended for the monoxenic culture of *P. zeae* but the method is laborious in terms of media preparation and ensuring nematode penetration into the roots (Meyer, 1984). Alfalfa callus tissue has also been used to culture *P. zeae* monoxenically, but it can result in low populations (Motalaote *et al.*, 1987). Sterile carrot discs offer a cost effective and relatively less laborious alternative for rearing nematodes, which can result in greater nematode multiplication compared with other methods (Speijer and De Waele, 1997). However, not all migratory plant-parasitic nematodes are suitable for rearing on carrot discs. For example, attempts to raise *Helicotylenchus multicinctus* (Cobb) Golden were unsuccessful (Speijer and De Waele, 1997). Therefore, the objective of this study was to assess the efficiency of sterile carrot discs for mass culturing of *P. zeae.*

3.2 Materials and methods

Fresh, thick and straight carrots (*Daucus carota* L.), cv. Nantes, purchased locally, were used for culturing. This cultivar was preferred because it was less succulent and less susceptible to rot during incubation compared to other cultivars. *Pratylenchus zeae* used for monoxenic culture were extracted from infected maize roots, obtained from farmers' fields in Iganga District, Uganda, using a modified Baermann's sieving method (Coyne *et al.*, 2007). The identification of the nematode as *P. zeae* was confirmed by Dr. Esther Van den Berg, Nematology Biosystematics Institute, Queenswood, South Africa. Nematodes were surface sterilised with streptomycin sulphate solution according to Speijer and De Waele (1997).

Preparation of the carrots followed procedures outlined by Speijer and De Waele (1997). Twenty live nematodes were transferred to the margins of each of the 40 sterile carrot discs contained in 3.5 cm diameter sterile glass Petri dishes (Moody *et al.*, 1973). All cultures were maintained in the dark at 25 ± 1℃, which is within the temperature range at which most *Pratylenchus* spp. reproduce successfully (Thames, 1982). The reproduction of the nematodes was assessed three months after inoculation, when the nematodes accumulated on the surface of most carrot discs and the Petri dish surface.

Nine out of 40 carrot discs became contaminated. From the remaining uncontaminated discs, 20 were selected randomly to assess final nematode populations. Nematodes from each disc were rinsed using 300 ml sterile water into separate beakers and specimens in three 2 ml aliquots per carrot disc were counted under a stereo-miscroscope. After rinsing, carrot discs were macerated separately in a kitchen blender for 7 seconds and nematodes further extracted using the modified Baermann's method over 24 hours. Nematode

suspensions from macerated carrots were reduced to 25 ml and nematodes in three 2 ml aliquots were counted.

3.3 Results

A mean density of 63 913 vermiform *P. zeae* were recovered from the surface of each carrot disc, and comprised 46% females, 54% juveniles and no males. The mean number of eggs recovered from the surface of each carrot disc was 37 892. Therefore, *P. zeae* females alone increased by a factor of 1 476, while the overall reproduction rate was of 5 090-fold following three months incubation at 25 ± 1^{o}C on carrot discs. The mean numbers of *P. zeae* recovered from each macerated carrot disc accounted for an additional 11 644 females, 8 738 juveniles and 7 469 eggs. Many of these appeared dead, however, and were therefore omitted from the final computations, but their presence indicates potential additional multiplication.

3.4 Discussion

Working with excised maize roots, Meyer (1984) recorded a *P. zeae* increase of 26.4-, 23.5- and 11.0-fold after incubation at 28 ^{o}C for two, three and six months, respectively. Incubation periods of longer than three months, therefore, may not necessarily result in higher production of *P. zeae*. Results presented here show that *P. zeae* reproduced on carrot discs at a multiplication rate far above the range recorded even for other root-lesion nematodes. Therefore, the use of carrot discs is recommended as a particularly suitable medium for culturing *P. zeae*.

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CHAPTER FOUR

Genetic analysis of resistance to nematodes in maize (*Zea mays* L.)

Abstract

Plant–parasitic nematodes especially *Pratylenchus zeae* and *Meloidogyne* spp. have been associated with grain yield loss in susceptible maize cultivars in Uganda. Knowledge of inheritance and combining abilities of existing germplasm would be important in breeding new lines with resistance to nematodes. This study was conducted to estimate general combining ability (GCA), specific combining ability (SCA) and genetic effects associated with nematode resistance in maize. The 30 F_1 hybrids generated from a 6 x 6 diallel and two local checks were evaluated in three sites in an 8 x 4 α-lattice design with three replications. From the results, GCA was more important for the reduction of *P. zeae* and *Meloidogyne* spp. densities, and increase of root mass, with a contribution of 72 to 93% of the phenotypic variance. Inbreds MP709 and CML206 had the highest GCA for *P. zeae* resistance whereas for grain yield, CML444, CML312 and CML395 had the highest GCA. The SCA was important for an increase in plant height and grain yield, contributing 43% and 58% of the phenotypic variance, respectively, under nematode infestation. Hybrids MP709/CML444 and MP709/CML395 had significant negative reciprocal effects for grain yield resulting from the negative maternal effects observed in parent MP709; an indication of greater susceptibility to nematodes in the two hybrids when MP709 is used as the female parent. Overdominance gene action seemed to explain the non-additive variance recorded for plant height, grain yield, number of root lesions, *P. zeae* and *Meloidogyne* spp. densities recorded under nematode infestation. Parents MP709, CML206, 5057 and CML444 contributed most of the dominant genes for *P. zeae* resistance in all of their crosses. Parent CML444 contributed most of the dominant genes towards improved grain yield in all of its crosses. Average heterosis was positive and in the desired direction for all traits evaluated except for *Meloidogyne* spp. densities. The high GCA effects among some parents supports breeding for widely adapted nematode resistant cultivars. Preponderance of dominant genes and SCA effects would favour pedigree breeding and various sib tests to improve grain yield under nematode pressure.

Keywords: General combining ability, Genetic effects, Grain yield, Maize, *Meloidogyne* spp., Specific combining ability**,** *Pratylenchus zeae*.

4.1 Introduction

Maize production is faced with many biotic challenges in the tropics such as turcicum leaf blight (TLB), maize streak virus (MSV), grey leafspot (GLS) and stem borers. The majority of these challenges have been or are being addressed in Uganda (Pratt *et al.*, 2003). However, the nematode challenge has for long not been given due consideration although studies have indicated yield losses, as high as 50%, which are associated with nematode damage on maize (Keetch, 1989; Bridge, 1994). The most economically important groups of plant-parasitic nematodes to maize production are the root-knot nematodes (*Meloidogyne* spp.), the root lesion nematodes (*Pratylenchus* spp.) and the cyst nematodes (*Heterodera* spp.) (McDonald and Nicol, 2005). In Uganda, the most prevalent nematode species on maize are *P. zeae* and *Meloidogyne* spp., respectively (Butseya *et al.*, 2005; Talwana *et al.*, 2008; Kagoda *et al.*, 2010).

Pratylenchus zeae is a migratory endoparasite of the root cortex, which enters the smaller roots at any point. *Pratylenchus zeae* particularly causes a mechanical breakdown of cells and necrosis of stellar and cortical tissues, resulting in formation of cavities (Olowe and Corbett, 1976; Olowe, 1977). On the other hand, *Meloidogyne* spp. young vermiform larvae penetrate the roots of plants near the growing points, migrate towards the stele and become sessile inside the root as adult females. Infection of maize roots by *Meloidogyne* spp. is characterised by typical multinucleated giant cell development in vascular tissue as well as embedded egg masses in inconspicuous galls, mostly close to root apexes (Asmus *et al.*, 2000). Generally, the major above-ground symptoms of nematode damage on maize are stunting and chlorosis, while the major below-ground symptom is root necrosis (due to *P. zeae*). Root galls (due to *Meloidogyne* spp.) are rare in maize but this does not indicate an absence of *Meloidogyne* spp. (Idowu, 1981; Riekert, 1995; Asmus *et al.*, 2000).

For nematode control in maize, host resistance is advocated because it is cheap, and poses no technical difficulties to the farmer, provided that resistance genes are readily available (Trudgill, 1991). McDonald and Nicol (2005) pointed out that pedigree breeding without selecting for nematode resistance in maize is likely to result in highly susceptible and intolerant crops, which could be very costly in any kind of production system. However, most of the maize breeding work across the globe has focused on the root-knot nematodes. This is because it is easier to identify resistance to sedentary than migratory endo-parasites (Jordaan and De Waele, 1987). However, the gene action conditioning resistance to nematodes of maize is not known. Similarly, no information exists on the combining abilities of maize inbred lines adapted to tropical conditions in Uganda, under nematode pressure. This information would be useful in selecting lines for hybrid development, and in choosing

the appropriate breeding procedure. Nevertheless, some nematologists have screened maize genotypes for resistance to *P. zeae*, and have been able to identify some resistant genotypes (Kimenju *et al.*, 1998; Oyekanmi *et al.*, 2007). This can form the basis to initiate studies to determine the genetics of resistance to nematodes and later be able to incorporate this resistance in elite lines.

The major objectives of this study were to:
Characterise the genetics of nematode resistance in maize, through:

- Estimating the general combining ability (GCA) of selected parents, and the specific combining ability (SCA) of a parent in a cross with another parent;
- Determining the contribution of cytoplasmic effects to inheritance of resistance to nematodes;
- Estimating the genetic effects which control the inheritance of nematode resistance in maize.

It was hypothesized that:

- Maize inbred lines have good GCA associated with nematode resistance and high grain yield;
- Maternal effects are important in the inheritance of resistance to nematodes in maize;
- Adequate genetic variation, incorporating both additive and dominance, is involved in conditioning resistance to nematodes in maize.

4.2 Materials and methods

4.2.1 Germplasm sources and selection criteria

Nine maize inbred lines with multiple trait resistances and susceptibilities were collected from diverse sources for evaluation and possible use in the diallel cross. Field trials were then conducted to establish the status of the inbred lines in terms of vigour, pest and disease resistance and grain yield (Appendix 4.1). Following the evaluation trials, six inbreds were selected for use in the diallel cross (Table 4.1).

Table 4.1: Parent inbred lines used in the diallel mating scheme

Parental line	Selection criteria/Main features	Source	Adaptation	Reference
MP709	*Meloidogyne* spp. & *P. zeae* resistant	USA, Mississippi	Temperate	Williams and Windham (1998)
5057	*P. zeae* resistant, striga susceptible,	IITA-Nigeria	Tropical/West Africa	Oyekanmi *et al.* (2007)
CML206	*P. zeae* resistant, susceptible to TLB & rust. MSV resistant	CIMMYT	Mid-altitude/subtropical	(Diallo *et al.*, 2001)
*CML444	Drought tolerant, IR, *P. zeae* tolerant, Low N tolerant, MSV & TLB resistant	CIMMYT	Mid-altitude/Subtropical	CIMMYT (2001)
CML395	Drought tolerant, IR, Low N tolerant, MSV & TLB resistant	CIMMYT	Mid-altitude/Sub-tropical	CIMMYT (2001)
CML312	Drought tolerant, IR, GLS & TLB resistant. MSV susceptible	CIMMYT	Subtropical	CIMMYT (2001)

IR = Imidazolinone resistant; N = Nitrogen. *Inbred CML444 expressed resistance to *Meloidogyne* spp. and tolerance to *P. zeae* in the preliminary screening trials of this study (Appendix 4.1).

4.2.2 Diallel crosses and evaluation of the parents and F_1 progeny

The six selected parents were crossed in the field at IITA-Namulonge farm using the 6 x 6 diallel mating design with reciprocal crosses. The parents, hybrid F_1s and reciprocals were evaluated under natural nematode infestations in the field. The evaluation trials were conducted at Namulonge (1 200 m.a.s.l); 0°32'N, 32°34'E; 1 300 mm bimodal rainfall), Kabanyolo (1 150 m.a.s.l; 0° 28'N, 32° 37'E; 1 300 mm bimodal rainfall) and Bufulubi (1 130 m.a.s.l; 00° 49' N, 033° 42' E; 1345 mm bimodal rainfall), which are characterized by greater than 40% sandy soils (Wortmann and Eledu, unpublished data; Appendix 4.2).

Field areas that had previously been densely planted with maize were used for the field screening trials. This is because maize roots which remain in the soil after harvest, as well as weeds and the soil itself, are a reservoir for nematodes and their eggs during the dry season. This is an important source of infestation for the early season crop (Egunjobi and Bolaji, 1979). At planting, soil samples were collected per plot and taken to the laboratory for nematode extraction and counting. Where nematode populations in the soil (P_i) were found low (< 500 *P. zeae* and < 100 *Meloidogyne* spp. per 100 g of soil) in the nematode unprotected plots, nematode infested maize roots were chopped into small pieces and applied per plant in the affected block. For example, in each of the sites used at Namulonge and Kabanyolo, 500 *P. zeae* and 70 *Meloidogyne* spp. were applied per plant in form of chopped root pieces in order to raise the nematode population in the soil to 600. Nematode infested maize roots were collected from pots and from nematode infested on-station field trials for use as inocula. To estimate the number and species of nematodes, the composite sample of roots collected were chopped, mixed thoroughly and weighed. Ten sub-samples

each weighing 5 g were then randomly picked from the composite sample and nematodes extracted from each following procedures outlined by Coyne *et al.* (2007). Nematode counts were then conducted per species under a stereomicroscope from each of the 10 sub-samples. It is from these sub-samples that the average number of nematodes per 5 g of root was obtained and later used to estimate the mass of root pieces containing the required number of nematodes for inoculation per plant.

Genotypes evaluated at the three sites were the 30 F_1 hybrids (including reciprocals), the six parents, and two local hybrids associated with high yield potential in Uganda, namely DK8031 (*P. zeae* resistant) and H614D (*P. zeae* susceptible). Therefore, the total number of genotypes evaluated was 38. The parents, being inbred lines, were evaluated in separate replicated blocks in a split plot design with nematode treatments (nematode infested and nematicide treated plots) serving as whole plots and the genotypes as subplots but arranged in a 3 x 2 alpha (0, 1) lattice design (Appendix 4.3). The 32 hybrids were also evaluated in a split plot design with nematode treatments serving as whole plots and the hybrids as subplots but arranged in an 8 x 4 spatially adjusted α-lattice design (Appendix 4.4). The experiments for inbreds and hybrids were adjacent to each other to avoid genotype x environmental complications. Two replications were established at Namulonge and three replications at Kabanyolo and Bufulubi for the parents and hybrid experiments. For both the parents and hybrids experiments, two-row plots were planted per genotype consisting of 32 plants. Inter and intra-row spacing was maintained at 0.75 m and 0.30 m. Prior to planting maize, soil samples were taken from a depth of 15 cm from each demarcated plot for nematode counts. The recommended amounts of phosphorus for maize production in Uganda were applied at planting, that is, 7.5 kg N ha^{-1} and 19.2 kg P_2O_5 ha^{-1} in the form of Di-ammonium phosphate (DAP). Fenamiphos (non-volatile) nematicide (commercially called Nemacur™) was applied at a rate of 2.5 kg ha^{-1} (2.3 g/0.45 m^2, which is the area surrounding the plant) and incorporated 5 to 8 cm deep with a hand hoe prior to planting in the treated (no nematode) plots (Rhoades, 1979; Taylor *et al.*, 1999). No evidence of phytotoxicity symptoms have been associated with fenamiphos (Rhoades, 1979). Two seeds were sown per hill and later thinned to one plant. Other standard agronomic practices such as hand weeding were followed at all the sites as recommended for the growers.

4.2.3 Data collection

4.2.3.1 Nematode damage and population density assessment

Soil samples were collected from each plot for counting the initial populations (P_i) of nematodes (vermiform) by species at or shortly before planting as suggested by Jordaan *et al.* (1989). The P_i was assessed from soil samples collected in each of the two rows (plot)

using a trowel to a depth of 15 cm, discarding the upper 5 cm (Todd and Oakley, 1996; Coyne *et al.*, 2006). About 10 soil sub-samples were taken and then mixed to form one sample per plot. From 50% flowering, roots were sampled from the root system of 10 randomly selected plants in each plot for final nematode population (P_f) assessment. In the laboratory, the roots were washed clean with tap water and their fresh mass recorded. Root lesions (black necrotic spots on surface of root) were then counted visually per 5 g of fresh roots. Nematodes (P_i) were extracted from a 100 ml soil sub-sample, and from a macerated 5 g fresh root mass (frm) sub-sample (P_f), using a modified Baermann sieve method (Coyne *et al.*, 2007). The samples were examined after a 48 hour extraction period, and nematodes counted using a stereomicroscope. Both P_i and P_f were estimated by taking the average of nematodes in three x 2 ml aliquots (taken from a 25 ml suspension).

4.2.3.2 Assessment of other agronomic traits and yield

Plant height (cm) was measured after all the plants had flowered as the distance from the base of the plant to the height of the first tassel branch (Magorokosho *et al.*, 2007). The maize was harvested when the moisture content of grain had declined to approximately 16% (Johnson *et al.*, 1999). Grain yield (t ha^{-1}), which is measured as grain mass and number of ears per plot, was taken on an entire plot basis at harvest and later adjusted to 12.5% moisture content (CIMMYT, 1985).

4.2.4 Statistical analysis

Data were tested for normality using the Proc Univariate Normal Plot procedure in SAS statistical package. Consequently, plant height, root mass, and number of root lesions were sqrt(x+10) transformed. The *P. zeae* and *Meloidogyne* spp. densities in the root were standardized by subtracting P_i from P_f per plot, i.e., the $P_f - P_i$ values were the nematode populations used in all analyses (Forrest and Holliday, 1979), measured per 100 g of fresh root mass (frm). The nematode densities were then log(x+10^3) transformed and grain yield was sqrt(x) transformed.

Genetic analyses for nematode resistance were performed as a fixed effects model for the 36 entries (30 experimental hybrids and the six parents) across the three sites. Diallel-SAS05 program was used to perform Griffing's method 1 diallel analysis (Zhang *et al.*, 2005). The analysis was based on the following model:

$Y_{ijkl} = \mu + S + g_i + g_j + s_{ij} + r_{ij} + g_iS + g_jS + s_{ij}S + r_{ij}S + e_{ijkl}$. Where:

Y_{ijkl} = value of F_1 cross of the i^{th} female and the j^{th} male in the k^{th} block and l plot/observation

μ = population mean;

i = j = 1, 2, …, n (n = 6 parents);

k = 1, 2, ..., b (b = 2 and 3 replications);

l = 1, 2, …, c (c = 32 plants per plot);

S = Site main effects;

g_i & g_j = GCA effects of i^{th} and j^{th} inbred parent, respectively;

s_{ij} = SCA effect ($s_{ij} = s_{ji}$);

r_{ij} = reciprocal effects ($r_{ij} = -r_{ji}$);

$g_i + g_j + s_{ij}$ is the genotypic contribution for cross i x j;

g_iS = GCA of i^{th} parent x site interaction effect;

g_jS = GCA of j^{th} parent x site interaction effect;

$s_{ij}S$ = SCA x site interaction effects;

$r_{ij}S$ = Reciprocal x Site interaction effects;

e_{ijkl} = error effect of the $ijkl^{th}$ observation.

The GCA:SCA ratio was calculated as $\frac{2MS_{GCA}}{2MS_{GCA} + MS_{SCA}}$ where MS = mean square (Baker, 1978). This ratio indicates the relative importance of heritable additive genetic effects against the non-additive (dominant) genetic effects.

The Hayman and Jinks analysis (Jinks and Hayman, 1953; Hayman, 1954) was performed using Genstat statistical software (Payne *et al.*, 2006) for only the data under nematode infestation. A test for homoscedasticity of error variance was conducted before pooling data across sites. The graphical approach (Hayman, 1954) was applied to test: (i) the adequacy of the dominance-additive model; (ii) the degree of dominance, i.e., whether partial, complete or overdominance; (iii) the direction of the dominance, in regard to prevalence of recessive over dominant genes. The equation for the model analysed in Genstat was:

Y = μ + site + a + b + c + d + a*site + b*site + c*site + d*site, where:

Y = observed effect;

μ = grand mean;

site = site effects;

a = additive effects;

b = dominance effects;

c = additive maternal effects;

d = maternal interaction effects.

Then a*site + b*site + c*site + d*site = total interaction of sites with the gene action components. The dominance effects (b) were further partitioned into:

b1, indicates direction of dominance. This term tests the mean deviation of the crosses from their mid-parent values, and it is significant only if the dominance deviations of the genes in the various entries used are predominantly in one direction;

b2, tests asymmetry of alleles. This term tests whether the mean dominance deviation of the crosses from their mid-parent values within each array differs over arrays. It will do so if some parents contain considerably more dominant alleles than others;
b3, tests whether some dominance is peculiar to some crosses.

The error term b*site is therefore also further partitioned into: site*b1, site*b2 and site*b3 to cater for the interaction of the sites with the dominance effects. Only significant genotypic differences allow the use of Hayman-Jinks model of genetic analysis (Hayman, 1954). The F-test was hence performed to test for significance, as the ratio of the mean square (MS) of a term in the model and the term*site interaction mean square, e.g., to test the additive effects, $F= MS_a/MS_{a*site}$, where a = additive effects.

A scaling test known as regression coefficient (b) analysis was used to further test the adequacy of the genetic model to describe the data set (Hayman, 1954). The regression coefficient was generated from a plot of the covariance (Wr) of family means with non-recurrent parents against the variance (Vr) of the family means within an array. Departure of b from zero was tested using $(b-0)/s.e._b$, whereas departure of b from 1 was tested using $(1-b)/s.e._b$, where s.e. = standard error. The genetic model was only considered adequate if the regression coefficients deviated significantly from zero but not from unity. This is possible if all the assumptions underlying the genetic model are fulfilled (Hayman, 1954). These assumptions (Hayman, 1954) are: (i) diploid segregation; (ii) no differences between reciprocal crosses; (iii) independent action of non-allelic genes, and in the diallel cross; (iv) no multiple allelism; (v) homozygous parents; and (vi) genes independently distributed between the parents. These were further taken into account by using diploids, accounting for significant reciprocal effects in the model, and using genetically diverse inbred lines as parents. Results obtained in the current study are restricted to the sample used.

Analysis of variance for effect of arrays on parental order of dominance (Wr+Vr) and the difference over the arrays (Wr-Vr) was performed using Proc GLM procedure in SAS, to determine the significance of epistasis.

4.3 Results

4.3.1 Combining ability effects

Significant ($P < 0.001$) site effects were detected for all traits measured under nematode infestation (Table 4.2). Similarly, the entries (hybrids and their parents) were significant ($P < 0.001$) for all the traits measured. General combining ability (GCA) was significant ($P < 0.05$) for all traits measured. Specific combining ability (SCA) was significant ($P < 0.05$) for plant

height, root mass, *Meloidogyne* spp. densities and grain yield. Site x Entry and Site x GCA interactions were significant for number of root lesions ($P < 0.05$) and grain yield ($P < 0.01$). Site x SCA interaction was significant ($P < 0.01$) for number of root lesions, *P. zeae* and grain yield. Reciprocal x Site, Site x Maternal and Non-maternal x Site interaction were not significant for all traits measured. The GCA:SCA ratio ranged from 0.42 (grain yield*)* to 0.93 (*P. zeae* densities). Therefore, SCA contributed 7 to 58% of the total phenotypic variance, with the highest (58%) contribution recorded for grain yield.

Under nematicide treatment, the sites had high significant ($P < 0.001$) effects on all traits of plant height, root mass and grain yield (Table 4.2). The Entries similarly had high significant ($P < 0.001$) effects on all the three traits. General combining ability (GCA) and specific combining ability (SCA) were highly significant ($P < 0.001$) for all traits measured. Site x Entry and Site x SCA interactions were highly significant ($P < 0.001$) for grain yield. Based on GCA:SCA ratio, SCA contributed 33 - 58% of the total phenotypic variance, with the highest contribution of the SCA recorded for grain yield.

Positive GCA effects for plant height and root mass are desirable (Table 4.3). Parent CML206 had negative and significant ($P < 0.05$) GCA effects for plant height whereas the rest of the parents had no significant GCA effects for plant height under nematode pressure. Parent MP709 had a negative and significant ($P < 0.05$) GCA effect for root mass unlike CML444 whose GCA was positive and significant ($P < 0.05$) for the same trait. Negative GCA effects for number of root lesions and nematode densities are desirable. All six parents did not have significant GCA effects for number of root lesions therefore the data is not presented. Parents MP709 and CML206 had negative and significant ($P < 0.01$) GCA effects for *P. zeae*. Conversely, parent CML312 had positive and significant ($P < 0.05$) GCA effect for *Meloidogyne* spp. For grain yield, parents MP709, 5057 and CML206 expressed negative and significant ($P < 0.05$) GCA effects, whereas parents CML444, CML395 and CML312 had positive and significant ($P < 0.05$) GCA effects.

Table 4.2: Mean squares for grain yield and other traits of maize diallel cross evaluated under nematode infestation and nematicide treatment over three sites

Source of variation	DF	Plant height	Root mass	Root lesions	*P. zeae*	*Meloidogyne* spp.	Grain yield
Nematode infested conditions							
SITE	2	635.7***	97.33***	133.5***	1.732***	0.290***	2.659***
REP(SITE)	5	5.783	14.63	8.49	3.592	0.493	0.489
†ENTRY	35	6.910***	2.74***	1.03***	0.220***	0.031***	2.421***
GCA	5	8.777***	6.255***	1.400**	0.918***	0.043**	1.874***
SCA	15	13.48***	4.313***	1.650	0.146	0.034**	5.207***
REC	15	1.085*	1.074	0.171	0.047	0.017	0.291**
MAT	5	2.458***	1.711	0.051	0.030	0.018	0.373*
NMAT	10	0.399	0.756	0.231	0.055	0.016	0.249*
SITExENTRY	70	0.630	0.80	0.58*	0.135	0.016	0.273***
SITExGCA	10	0.937	1.195	0.801*	0.087	0.014	0.359**
SITExSCA	30	0.631	0.667	0.899***	0.201**	0.019	0.365***
SITExREC	30	0.527	0.799	0.190	0.086	0.013	0.152
SITExMAT	10	0.894	0.869	0.086	0.096	0.011	0.117
SITExNMAT	20	0.343	0.764	0.241	0.081	0.013	0.170
GCA:SCA ratio		**0.57**	**0.74**	**0.63**	**0.93**	**0.72**	**0.42**
Nematicide treated conditions							
SITE	2	953.4***	95.4***				1.45***
REP(SITE)	5	68.9	4.6				0.81***
ENTRY	35	13.9***	3.4***				1.95***
GCA	5	33.2***	3.964***				1.55***
SCA	15	32.4***	7.168***				4.32***
REC	15	3.8	0.678				0.11
MAT	5	3.0	0.395				0.13
NMAT	10	4.1	0.820				0.10
SITExENTRY	70	4.4	0.9				0.27***
SITExGCA	10	7.8	0.441				0.16
SITExSCA	30	1.8	0.740				0.34***
SITExREC	30	2.5	0.580				0.14
SITExMAT	10	2.4	0.631				0.13
SITExNMAT	20	2.5	0.555				0.15
GCA:SCA ratio		**0.67**	**0.53**				**0.42**

REP = Replication; SITE = Environmental effect; MAT = Maternal effects; NMAT = Non-maternal effects; REC = Reciprocal effects. Melo = *Meloidogyne* spp. *, **, and *** represent significance at P ≤ 0.05, P ≤ 0.01 and P ≤ 0.001. †ENTRY used in reference to the 30 hybrids and the six parents.

Table 4.3: General combining ability effects of parents for grain yield and other traits across sites

Parent	Plant height (cm)	Root mass (g)	*P. zeae* (per 100g frm[1])	*Meloidogyne* spp. (per 100g frm)	Grain yield (t ha^{-1})
Nematode infested conditions					
MP709	0.180	-0.287*	-0.123**	-0.012	-0.173***
5057	-0.337	-0.227	0.030	-0.005	-0.094*
CML206	-0.422*	0.166	-0.117**	-0.028	-0.104**
CML444	0.234	0.289*	0.039	0.019	0.152***
CML395	0.076	-0.169	0.052	-0.005	0.131**
CML312	0.269	0.228	0.119**	0.031*	0.088*
Nematicide treated conditions					
MP709	0.846*	-0.253			-0.139**
5057	-0.776*	-0.181			-0.039
CML206	-0.367	0.161			-0.154***
CML444	-0.098	0.149			0.123**
CML395	-0.239	-0.134			0.040
CML312	0.634	0.258*			0.168***

[1]frm = fresh root mass. *, **, and *** represent significance at P ≤ 0.05, P ≤ 0.01 and P ≤ 0.001, respectively.

Under nematicide treatment, parent MP709 had positive and significant (P < 0.05) GCA effects, whereas parent 5057 had negative and significant (P < 0.05) GCA effects for plant height. For root mass, parent CML312 had a positive and significant (P < 0.05) GCA effect, whereas the rest of the parents were not significant. For grain yield, parents MP709 and CML206 had negative and significant (P < 0.01) GCA effects, whereas parents CML444 and CML312 had positive and significant (P < 0.01) GCA effects.

Under nematode infestation, hybrids MP709/CML312, 5057/CML312, CML444/CML312 and CML395/CML312 had significant SCA effects for plant height (Table 4.4). Root mass SCA effects were significant (P < 0.05) for 5057/CML312, CML206/CML395, CML206/CML312 and CML395/CML312. The SCA effects were not significant for number of root lesions and *P. zeae* densities in all hybrids hence the data is not presented. The SCA of hybrid CML444/CML395 was positive and significant (P < 0.05) for *Meloidogyne* spp. densities. For grain yield, positive SCA effects were obtained for all hybrids. Most hybrids also displayed significant (P < 0.05) SCA effects except MP709/5057, MP709/CML395, CML206/CML444 and CML444/CML395.

Under nematicide treatment, only hybrids MP709/CML312 and CML444/CML312 had significant (P < 0.05) SCA effects for plant height. Root mass SCA effects were significant (P

< 0.05) for MP709/CML312, 5057/CML312, CML206/CML312, CML444/CML312 and CML395/CML312. For grain yield, most hybrids had significant (P < 0.05) SCA effects except MP709/5057, 5057/CML206, 5057/CML312 and CML206/CML444.

Table 4.4: Specific combining abilities of F_1 hybrids for grain yield and other traits across sites

Hybrid	Plant height (cm)	Root mass (g)	*Meloidogyne* spp. (per 100g frm)	Grain yield (t ha^{-1})
Nematode infested conditions				
MP709/5057	0.206	-0.236	0.014	0.120
MP709/CML206	0.566	0.239	-0.004	0.233**
MP709/CML444	0.278	-0.028	0.044	0.250**
MP709/CML395	0.618	0.174	-0.041	0.148
MP709/CML312	2.354*	0.629	0.086	1.131***
5057/CML206	0.508	-0.127	0.036	0.269**
5057/CML444	0.489	0.163	0.051	0.248**
5057/CML395	0.453	0.340	-0.007	0.321***
5057/CML312	2.725**	1.276*	0.110	1.314***
CML206/CML444	0.324	0.413	-0.013	0.123
CML206/CML395	0.014	0.384*	-0.016	0.216*
CML206/CML312	1.827	1.282*	0.035	1.443***
CML444/CML395	0.001	0.405	0.067*	0.164
CML444/CML312	2.071*	0.930	0.049	1.321***
CML395/CML312	1.944*	1.422*	0.077	1.675***
Nematicide treated conditions				
MP709/5057	-0.34	0.014		0.174
MP709/CML206	1.189	0.378		0.234*
MP709/CML444	0.327	-0.017		0.264**
MP709/CML395	1.343	0.435		0.383***
MP709/CML312	6.118***	2.148***		0.263**
5057/CML206	0.467	0.08		0.143
5057/CML444	0.742	0.286		0.302**
5057/CML395	0.87	0.207		0.375***
5057/CML312	2.644	1.693**		0.139
CML206/CML444	0.177	0.506		0.118
CML206/CML395	0.218	-0.186		1.550***
CML206/CML312	2.801	1.737**		1.305***
CML444/CML395	0.225	0.527		1.620***
CML444/CML312	3.721*	1.698**		1.269***
CML395/CML312	2.339	1.219*		1.354***

*, **, and *** represent significance at P ≤ 0.05, P ≤ 0.01 and P ≤ 0.001, respectively.

Reciprocal differences

Under nematode infestation, reciprocal effects were significant ($P < 0.05$) for plant height and grain yield (Table 4.2). Maternal effects were also significant ($P < 0.05$) for plant height and grain yield. Non-maternal effects were significant only for grain yield. Negative and significant reciprocal effects for grain yield were recorded for hybrids MP709/CML444 (-0.242; $P = 0.0312$) and MP709/CML395 (-0.288; $P = 0.0104$). All the other traits had non-significant reciprocal effects for all the hybrids. None of the parents had significant maternal effects at $P = 0.05$ for plant height, root mass, root lesions and nematode populations. MP709 had negative and significant (-0.114; $P = 0.0064$) maternal effects for grain yield. None of the hybrids had significant non-maternal effects at $P = 0.05$ for plant height, root lesions and nematode populations. Hybrid MP709/CML312, however, had significant positive (0.199; $P = 0.0297$) non-maternal effects for grain yield.

Under nematicide treatment, reciprocal effects were not significant for all the traits measured and the data is therefore not discussed.

4.3.2 Genetic effects for resistance to nematodes

The traits initially presented for the Hayman diallel analysis, using data under nematode infestation, include plant height, root mass, number of root lesions, *P. zeae* densities, *Meloidogyne* spp. densities and grain yield. However, ANOVA indicated that root mass data was not significantly influenced by all the items in the model. Root mass data was hence dropped from ANOVA (Table 4.5) and subsequent analyses.

Analysis of variance of the diallel table indicated highly significant ($P < 0.001$) additive effects (a) controlling plant height and *P. zeae* densities under nematode infestation (Table 4.5). Additive effects were, however, not significant for the number of root lesions, *Meloidogyne* spp. densities and grain yield. Dominance effects (b) were highly significant ($P < 0.001$) for plant height, *P. zeae* densities and grain yield, and significant ($P = 0.05$) for the number of root lesions and *Meloidogyne* spp. densities. On partitioning of the dominance effects, direction of dominance (b1) was significant ($P < 0.05$) for only plant height and grain yield.

The *P. zeae* densities showed significance for asymmetry of alleles (b2) ($P < 0.05$) and residual dominance effects ($P < 0.001$). Maternal effects (c) were significant ($P < 0.05$) for plant height and grain yield whereas the non-maternal effects (d) were significant ($P < 0.05$) for only the *P. zeae* densities.

Table 4.5: Mean squares for grain yield and other traits associated with nematode resistance in maize under nematode infestation

Source	d.f.	Plant height (cm)	No. of root lesions	*P. zeae* (per 100g frm)	Melo (per 100g frm)	#Grain yield (t ha^{-1})
Site	2	265.1	58.188	0.05	0.56	1.48
Additive effects (a)	5	4.19***	0.46ns	0.41***	0.05ns	0.39ns
Dominance effects(b)	15	6.50***	0.74*	0.21***	0.03*	0.80***
b1	1	90.30**	8.25ns	0.96ns	0.17ns	11.02**
b2	5	0.42ns	0.36ns	0.20*	0.02ns	0.07ns
b3	9	0.57ns	0.11ns	0.14***	0.02ns	0.07ns
Maternal effects (c)	5	1.15*	0.02ns	0.04ns	0.02ns	0.12*
Non-maternal effects (d)	10	0.60ns	0.07ns	0.08*	0.02ns	0.10ns
Total (t)	35	3.72	0.40	0.18	0.03	0.45
Error terms						
Site x a	10	0.40	0.34	0.03	0.02	0.23
Site x b1	2	0.34	2.82	0.15	0.01	0.001
Site x b2	10	0.59	0.27	0.06	0.02	0.07
Site x b3	18	0.38	0.11	0.02	0.01	0.05
Site x b	30	0.45	0.35	0.04	0.02	0.05
Site x c	10	0.32	0.03	0.03	0.01	0.02
Site x d	20	0.58	0.09	0.03	0.01	0.09
Site x t	70	0.46	0.23	0.03	0.01	0.09

b1 = direction of dominance; b2 = asymmetry of alleles; b3 = residual dominance effects. # Two sites were used for grain yield.

Regression of covariance (Wr) on variance (Vr) for plant height, number of root lesions, *P. zeae* densities, *Meloidogyne* spp. densities and grain yield were based on data under nematode infestation (Figures 4.1-4.5).

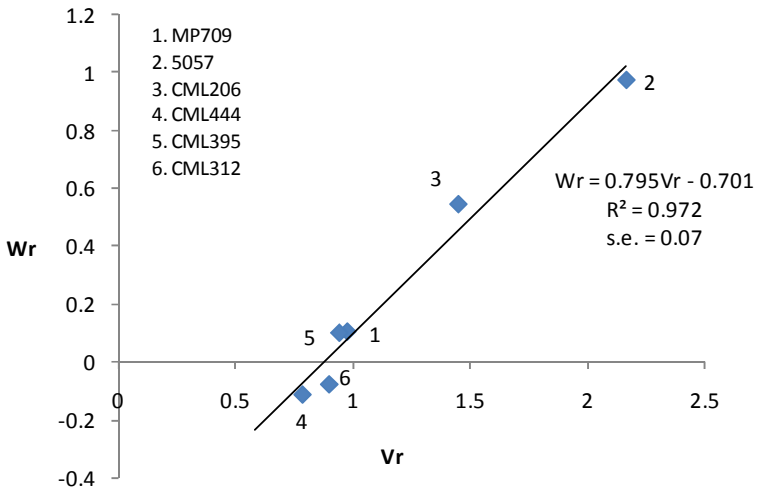

Figure 4.1: Linear regression of Wr/Vr for plant height

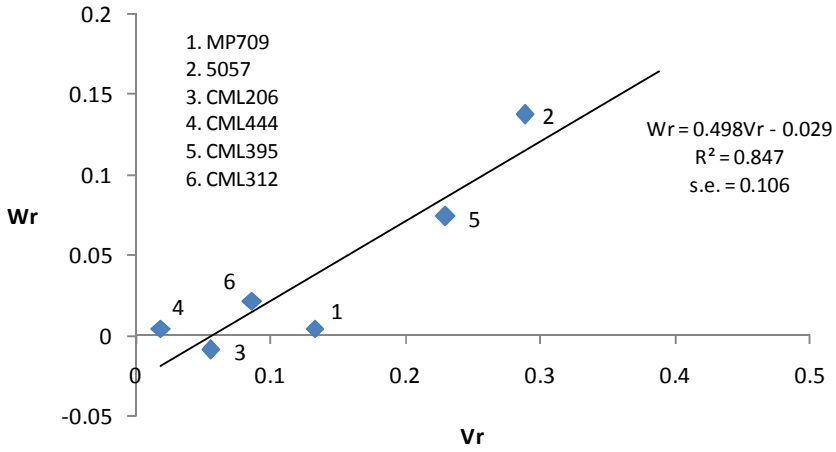

Figure 4.2: Linear regression of Wr/Vr for the number of root lesions

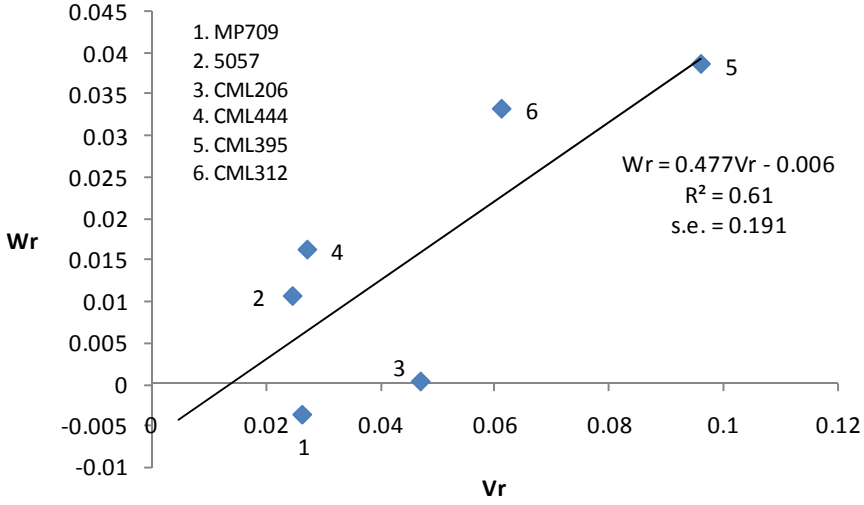

Figure 4.3: Linear regression of Wr/Vr for *P. zeae* densities

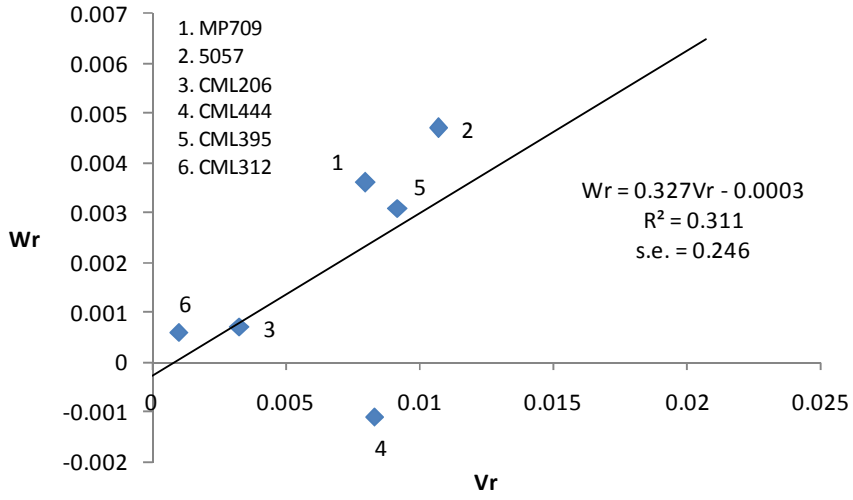

Figure 4.4: Linear regression of Wr/Vr for *Meloidogyne* spp. densities

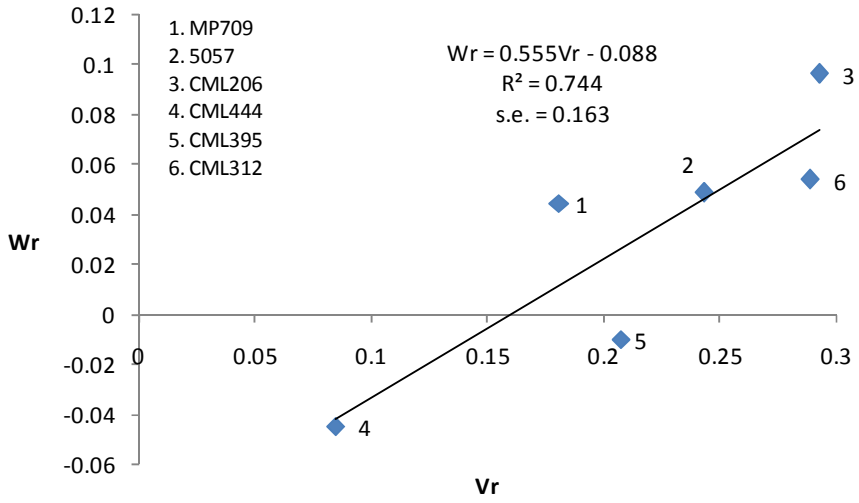

Figure 4.5: Linear regression of Wr/Vr for grain yield

All the array means were higher than the parent means over the six arrays for plant height (Table 4.6). The array means were highest in parents MP709, CML444 and CML312. These parents also displayed the lowest variances (Vr) and covariances (Wr). Parents CML206 and 5057 had high parental order of dominance (Wr+Vr) values but very low difference over the arrays (Wr-Vr) for plant height.

For number of root lesions, the six array means were lower than the parent means over the six arrays. Array means were lowest in MP709, CML206 and CML444. For *P. zeae*, only one array mean was higher than the corresponding parent mean over the six arrays. Parents MP709, CML206 and CML444 had the lowest array means and among the lowest Vr and Wr. CML395 and CML312 had the highest Wr+Vr values but the lowest Wr-Vr values for *P. zeae* densities. For *Meloidogyne* spp., only one array mean was lower than the parent mean over the six arrays. Within the array means, *Meloidogyne* spp. was lowest in MP709, 5057 and CML206 though CML395 also maintained quite lower populations. The Vr and Wr values were high in these parents. Parents CML395 and 5057 had the highest Wr+Vr values but the lowest Wr-Vr values. The average heterosis was positive, which is obviously not desirable for *Meloidogyne* spp. densities.

For grain yield, all the array means were higher than the parent means over the six arrays. The parents CML444 and CML395 had the highest array means for grain yield but with very low Vr and Wr. CML312 and CML206 had the highest Wr+Vr values but the lowest Wr-Vr values.

Table 4.6: Array means, variances (Vr) and covariances (Wr) for the traits associated with resistance to nematodes across sites

Array	Array mean	Parent mean	Vr	Wr	Wr-Vr	Wr+Vr
	Plant height					
MP709	12.291	10.4	0.973	0.103	-0.871	1.076
5057	11.835	8.92	2.163	0.972	-1.190	3.135
CML206	11.548	9.17	1.447	0.543	-0.904	1.990
CML444	12.318	10.5	0.783	-0.115	-0.898	0.668
CML395	12.260	10.6	0.939	0.099	-0.840	1.038
CML312	12.412	10.7	0.896	-0.080	-0.976	0.816
[+]Av. heterosis	2.45					
	Root lesions					
MP709	4.926	5.66	0.133	0.004	-0.129	0.137
5057	5.087	6.16	0.288	0.137	-0.151	0.425
CML206	4.899	5.28	0.056	-0.009	-0.065	0.047
CML444	4.933	5.11	0.019	0.004	-0.016	0.023
CML395	5.184	6.09	0.230	0.074	-0.156	0.303
CML312	5.058	5.50	0.087	0.021	-0.066	0.107
Av. heterosis	-0.742					
	P. zeae					
MP709	3.799	3.89	0.026	-0.004	-0.030	0.023
5057	3.942	4.07	0.025	0.011	-0.014	0.035
CML206	3.758	4.13	0.047	0.000	-0.047	0.047
CML444	3.887	3.79	0.027	0.016	-0.011	0.043
CML395	3.902	4.26	0.096	0.039	-0.058	0.135
CML312	4.059	4.48	0.061	0.033	-0.028	0.094
Av. heterosis	-0.253					
	***Meloidogyne* spp.**					
MP709	3.192	3.08	0.008	0.004	-0.004	0.012
5057	3.200	3.01	0.011	0.005	-0.006	0.015
CML206	3.167	3.11	0.003	0.001	-0.003	0.004
CML444	3.244	3.11	0.008	-0.001	-0.010	0.007
CML395	3.204	3.15	0.009	0.003	-0.006	0.012
CML312	3.263	3.27	0.001	0.001	0.000	0.002
Av. heterosis	0.107					
	Grain yield					
MP709	1.885	1.04	0.181	0.045	-0.136	0.226
5057	2.022	1.07	0.243	0.049	-0.194	0.292
CML206	1.885	0.80	0.292	0.096	-0.196	0.389
CML444	2.171	1.61	0.084	-0.045	-0.129	0.040
CML395	2.158	1.39	0.207	-0.010	-0.217	0.198
CML312	2.088	1.07	0.289	0.054	-0.235	0.343
Av. heterosis	1.050					

[+]Av. heterosis = Average heterosis or mean potence obtained as the mean of all crosses minus the mean of all parents for a given trait

Analysis of variance for effect of arrays (parents) on Wr+Vr and Wr-Vr values showed non-significant ($P > 0.05$) effects of Wr+Vr and Wr-Vr for plant height, number of root lesions, *Meloidogyne* spp. and grain yield (Table 4.7). For *P. zeae*, Wr-Vr had a significant ($P < 0.05$) effect but no significance was obtained at $P = 0.05$ for Wr+Vr.

Table 4.7: Mean squares for Wr-Vr and Wr+Vr values following analysis of variance for grain yield and other traits

Source	DF	Wr-Vr	Wr+Vr
Plant height			
Sites	2	1.009	0.970
Arrays (parents)	5	0.074ns	2.764ns
R^2 (%)		69.3	64.9
Root lesions			
Sites	2	0.095	0.312
Arrays	5	0.012ns	0.325ns
R^2 (%)		69.3	34.2
P. zeae			
Sites	2	0.011	0.002
Arrays	5	0.002*	0.004ns
R^2 (%)		84.0	57.9
***Meloidogyne* spp.**			
Sites	2	0.0008	0.003
Arrays	5	0.0001ns	0.0001ns
R^2 (%)		74.4	85.4
Grain yield			
Sites	1	0.001	0.000
Arrays	5	0.003ns	0.030ns
R^2 (%)		51.5	62.4

4.4 Discussion

4.4.1 Combining ability effects

The significance of sites for all traits (*P. zeae*, *Meloidogyne* spp., root mass, root lesions and grain yield) implies that there were phenotypic variations among genotypes associated with change in environment. Significant differences in all traits across entries suggest the presence of sufficient genetic variation for those traits that can be exploited through effective selection. Based on Baker (1978) ratios, GCA contributed most to increased root mass, and *P. zeae* and *Meloidogyne* spp. resistance, whereas it had less contribution to grain yield and plant height under nematode infestation. Williams and Windham (1992) similarly found GCA to be a significant source of variation for *Meloidogyne* spp. resistance. Therefore, single cross progeny performance is predictable based on the GCA of its parents (Falconer, 1981) for root mass, *P. zeae* and *Meloidogyne* spp. resistance.

The SCA was more important in determining performance of hybrids for grain yield and plant height than GCA under nematode pressure. The SCA effects were not significant for *P. zeae* and the number of root lesions, implying that GCA effects make the greatest contribution towards resistance to *P. zeae*. The GCA effects have also been reported to be predominant in the inheritance of *P. thornei* resistance in wheat in Australia (Zwart *et al.*, 2004). Zhang *et al.* (2007) found GCA to be more important than SCA in the inheritance of *M. incognita* resistance in upland cotton. The significant influence of reciprocal effects on plant height and grain yield suggests presence of cytoplasmic and nuclear genes interacting to affect the

expression of these traits under nematode infestation. This was not the case under nematicide treated plots.

The *P. zeae* and *Meloidogyne* spp. densities were not significantly influenced by site x GCA interaction unlike most of the other traits (Table 4.2). This implies that the parents with excellent GCA effects for *P. zeae* and *Meloidogyne* spp. resistance do not require a specific environment for these genes to be expressed. However, site x SCA interaction effects were significant for *P. zeae* densities, an indication that resistance to *P. zeae* based on SCA is differentially expressed in various environments. The significance of site x GCA and site x SCA interactions for grain yield under nematode infestation implies that different genotypes are adapted to specific environments, which poses a challenge for the development of widely adaptable cultivars of maize with resistance to nematodes.

4.4.1.1 General combining ability effects of parents

All parents did not have significant GCA effects for plant height except CML206 which had significant and negative GCA effects under nematode infestation. Therefore, CML206 is not a good general combiner for improving plant height under nematode pressure. Root mass had positive GCA effects in CML444, which suggests nematode tolerance in this parent. However, a negative GCA effect for root mass observed in the inbred MP709 indicates that MP709 impacted negatively on root mass in its hybrid combinations but only under nematode pressure. Reduction in root growth is a characteristic of *P. zeae* infection (Olowe and Corbett, 1976), but since MP709 is not adapted to the tropical environment, this might have negatively affected its ability to develop a normal root system. Nevertheless, MP709 and CML206 were the best general combiners associated with *P. zeae* resistance. Consequently, breeding methods that take advantage of additive genetic variance, such as the backcross and recurrent selection, can be used with MP709 and CML206 as sources of resistance, to increase the level of resistance to *P. zeae* in maize cultivars with good agronomic traits.

Parent CML312 had a positive and significant GCA effect for *P. zeae* and *Meloidogyne* spp. densities, which compromised resistance to nematodes in its crosses. According to Rasmann *et al.* (2005), nematode susceptible maize varieties have an abundance of (E)-b-caryophyllene in their roots which acts as a long-range attractant for nematodes. This may have been the case in CML312 though actual investigations are required. Inbreds MP709, 5057 and CML206 had negative GCA contributions to grain yield whereas CML444, CML312 and CML395 enhanced grain yield with positive GCA effects under both nematode infested and nematicide treated conditions. Inbred CML206 is inherently small seeded which may

have limited its contribution to grain yield despite being nematode resistant. Inbred lines MP709 and 5057 were imported from the temperate conditions of the USA and the tropical conditions of Nigeria, respectively, and did not adequately adapt to the tropical conditions in Uganda, with the consequent negative contribution to grain yield. The three inbreds can, however, be utilized in developing nematode resistant and high yielding hybrids by crossing them with adapted genotypes with large kernels.

4.4.1.2 Specific combining ability effects

Significant SCA effects for plant height in hybrids MP709/CML312, 5057/CML312, CML444/CML312 and CML395/CML312 is an indication that SCA contributed most in the inheritance of plant height in these hybrids under nematode infestation. The trend was, however, different under nematicide treatment with only hybrids MP709/CML312 and CML444/CML312 having significant SCA effects for plant height. However, Zsuzsanna *et al.* (2002) and Sibiya (2009) have reported plant height to be controlled by additive genes without taking nematodes into consideration. The SCA of hybrid CML444/CML395 was positive and significant for *Meloidogyne* spp. densities, indicating that this hybrid is susceptible to *Meloidogyne* spp. According to Windham and Williams (1988), and Williams and Windham (1992), both GCA and SCA are significant sources of variation in the inheritance of *M. incognita* resistance in maize.

The SCA effects for grain yield were positive and significant for 11 hybrids (with MP709/CML312, 5057/CML395, 5057/CML312, CML206/CML312, CML444/CML312 and CML395/CML312 having the highest SCA effects) under nematode infestation and nematicide treated plots. The majority of these hybrids with significant SCA for grain yield were similar with and without nematicide suggesting that the same genes (dominant genes) controlled the trait in each of the hybrids. Contradicting reports exist on whether grain yield in maize is controlled by additive genes, non-additive genes or both. Betrán and Hallauer (1996) reported that additive effects were more important than dominance for grain yield in hybrids. However, additive, dominance and epistasis effects have been reported to explain high yield in widely adapted hybrids (Wolf and Hallauer, 1997; Wolf *et al.*, 2000). Under drought conditions, Derera *et al.* (2007) reported GCA to significantly improve yield among southern African maize hybrids whereas both GCA and SCA were important under non-drought conditions. Sibiya (2009) recorded significant SCA effects towards reduced Phaeosphaeria leaf spot and high grain yield in maize and deduced that non-additive gene effects can be utilized in hybrid development. Results from the current study indicate SCA effects to offer more contribution to grain yield (58%) among hybrids under nematode

infestation, and this is associated with non-additive genes. Consequently, the role of SCA and GCA depends on the set of germplasm and the environment sampled.

The hybrids 5057/CML206 and MP709/CML206 had positive and significant SCA effects for grain yield despite being derived from parents with negative and significant GCA effects for the same trait under nematode infestation. This implies that poor general combiners can produce cross combinations with good SCA for traits controlled by overdominance genetic effects, which was the case for grain yield. For this reason, sum of all SCA effects was not equal to zero since it was a purely non-additive case. Dominance deviation SCA can only be equal to zero in a purely additive case (Kiekens *et al.*, 2006).

Hybrids MP709/CML444 and MP709/CML395 had negative and significant reciprocal effects for grain yield under nematode infestation. These can be explained by the negative maternal effects observed for grain yield in parent MP709 when used as a female parent. However, this might be due to the failure of the inbred MP709 to adequately adapt to the local environmental conditions, resulting in low yields in some of its hybrid combinations (Chapter 5). Notably, hybrid MP709/CML312 had positive and significant non-maternal effects for grain yield. Therefore, grain yield for this hybrid combination is influenced by the interaction of nuclear and cytoplasmic genes under nematode infestation.

4.4.2 Genetic effects

The additive-dominance model was adequate for determining the gene action for plant height, number of root lesions, *P. zeae* densities, *Meloidogyne* spp. densities and grain yield under nematode infestation. This is because their regression coefficients deviated significantly ($P \leq 0.05$) from zero but not from unity (Hayman, 1954). From the analysis of variance, both additive and dominant genes were important in the inheritance of increased plant height and reduced *P. zeae* densities on the maize genotypes under nematode infestation. However, dominant genes played a major role in the inheritance of plant height whereas additive genes were more important in the inheritance of *P. zeae* resistance, based on the magnitudes of the respective mean squares. Sawazaki *et al.* (1987) reported dominant genes to be responsible for resistance to *P. zeae* and *P. brachyurus* in a maize field naturally infested with both nematode species. In the current study, dominance effects mainly controlled the inheritance of reduced root lesions, *Meloidogyne* spp. densities, and increased grain yield under nematode pressure. This did not corroborate with findings by Williams and Windham (1992) that additive genes are a significant source of variation for *Meloidogyne* spp. resistance. Zhang *et al.* (2007) reported partial dominance effects to control inheritance of *Meloidogyne incognita* resistance in upland cotton.

For plant height and grain yield, the dominance was largely unidirectional since it was significant for b1. The array means were higher than the parental means for both of these traits, which indicates that dominance is in the direction of tall plants with high grain yield. Similarly, Stuber (1994) reported increased plant height in F_1 hybrids to be due to complementary effects of dominant alleles at two loci, one affecting node number and the other affecting internode length leading to heterosis. Likewise, dominant genes and epistasis affect the improvement of grain yield since they are the genetic basis of heterosis (Falconer, 1981).

Asymmetry in gene distribution (b2) was significant for *P. zeae* densities. Therefore, some parents namely MP709, 5057, CML206 and CML444 contained more dominant alleles for *P. zeae* resistance than CML395 and CML312. Residual dominance effects (b3) were also significant for *P. zeae* densities, which confirms that some dominance for *P. zeae* resistance is peculiar to individual F_1 crosses, resulting from epistasis or failure of assumptions.

Presence of maternal effects for grain yield confirms results obtained earlier in the current study using Griffing's analysis. The maternal effects obtained in Griffing's analysis were a result of inbred line MP709, which led to greater nematode susceptibility when used as a female parent in two of the crosses. The non-maternal effects for *P. zeae* densities observed in Hayman analysis imply that nuclear and cytoplasmic genes are interacting in the inheritance of *P. zeae* resistance.

The Wr/Vr regression gives a measure of the adequacy of the model, average dominance, and the distribution of dominant and recessive genes (Hayman, 1954). For plant height, Wr/Vr regression over the three sites was significantly ($P < 0.001$) different from zero with a regression coefficient not significantly different from unity. All the parents were closer to the regression line, an indication of absence of epistatic effects. The regression line intercepted the Wr axis far below the origin, which signifies that overdominance effects were important in the inheritance of plant height under nematode pressure. Parents MP709, CML395, CML312 and CML444 contributed most dominant genes for plant height since they were clustered closer to the origin of the regression line (Fig. 4.1). The dominant genes were specifically associated with an increase in plant height which is reflected by the large array means compared to the parental means. These parents also had low variances and covariances, a characteristic of dominant alleles (Hayman, 1954; Kearsey and Pooni, 1996). Parent CML206 was in the middle and 5057 at the extreme end of the regression line. Therefore, parent CML206 contributed both dominant and recessive genes whereas parent 5057 had

recessive genes associated with reduced plant height under nematode pressure. The very low Wr-Vr value but high Wr+Vr value for parent 5057 was further confirmation of recessive genes controlling inheritance of reduced plant height in this parent.

Overdominance was similarly observed for the number of root lesions, *P. zeae* densities and *Meloidogyne* spp. densities since the respective regression lines were below the origin. However, for *Meloidogyne* spp., the regression coefficient was not significantly different from zero and had a low R^2 value hence the data was not statistically valid at P = 0.05. Nevertheless, parents CML312 and CML206 contributed most of the dominant genes for susceptibility and resistance to *Meloidogyne* spp., respectively. Lordello and Lordello (1992) attributed resistance to *M. javanica* to dominant genes prevalent in IAC Ip365-4-1 maize parental line. Parents MP709 and CML395 contributed both dominant and recessive genes with MP709 favouring resistance whereas CML395 genes were inclined towards susceptibility to *Meloidogyne* spp. Parent 5057 contributed most of the recessive genes associated with susceptibility to *Meloidogyne* spp. However, since parent CML444 deviated highly from the regression line it probably has epistatic genes associated with susceptibility to *Meloidogyne* spp. According to Jink (1954), high deviation of some genotypes from the slope of one for a particular trait is a result of genic interactions.

For the number of root lesions, the regression coefficient was significant at P = 0.005. Parents MP709, CML206 and CML444 contributed most of the dominant genes towards reduced number of root lesions since they were closer to the origin, whereas parent CML395 contributed both dominant and recessive genes (Fig. 4.2). Parent 5057 had most of the recessive genes since it was furthest from the origin. However, this contradicts earlier findings by Oyekanmi (2007) that this parent is resistant to *P. zeae* since root lesions are a result of *P. zeae* damage. Probably the tropical conditions in Uganda had a negative impact on performance of this parent in terms of number of root lesions. Nevertheless, all the parents were closer to the regression line, which indicates absence of epistatic effects. The six array means for number of root lesions were lower than the parent means over the six arrays, which was further proof that genes for enhancing reduced number of root lesions were dominant over genes for susceptibility.

For *P. zeae* densities, regression of Wr/Vr was significantly different (P = 0.05) from zero. Parent MP709, 5057, CML206 and CML444 contributed most of the dominant genes towards resistance since they were closer to the origin of the graph, whereas CML312 and CML395 had most of the recessive genes associated with susceptibility to *P. zeae* (Fig. 4.3). Resistance to *P. zeae* by parent 5057 corroborates findings by Oyekanmi (2007) whereas

resistance to *P. zeae* by MP709 and CML206 is consistent with findings from preliminary screening studies in the current study (Table 4.1; Appendix 4.1). Parent CML444 was tolerant to *P. zeae* in the preliminary studies (Table 4.1; Appendix 1). The preliminary studies were, however, based on a single site compared to the final evaluation trials (three sites), which might be the reason for the differences in ranking.

Grain yield inheritance under nematode pressure was controlled by overdominance gene effects since the regression line intercepted the Wr axis far below the origin (Fig. 4.5). Nawar *et al.* (1997) similarly recorded the presence of overdominance in the inheritance of grain yield and other traits such as ear length and plant height, but not under nematode pressure. On the graph, the array points were scattered along the regression line which indicates genetic diversity among the parents for grain yield. Parent CML444 contributed most dominant genes for high grain yield, whereas MP709, CML395 and 5057 had similar frequency of both dominant and recessive genes. Parents CML206 and CML312 had most recessive genes for reduced grain yield under nematode pressure. Nearness of parents CML444 and 5057 to the regression line may suggest that these two parents were entirely free from non-allelic interaction and linkage. For grain yield, all the array means were higher than the parent means over the six arrays, suggesting that genes for high grain yield were dominant over genes for low grain yield.

Average heterosis was positive and in the desired direction for plant height and grain yield, which supports the preponderance of overdominance and dominance genes in the inheritance of these traits. For *P. zeae* and number of root lesions, average heterosis was negative, which is in the desired direction. There is no information on heterosis for *P. zeae* and *Meloidogyne* spp. resistance in maize. However, heterosis for root-knot nematode resistance has previously been reported in four tomato hybrids (Rani *et al.*, 2009). According to Betrán (2003), heterosis has been observed to be generally greater under stress conditions than under non-stress conditions. This indicates that hybrid vigour can be achieved among hybrids improved for resistance to *P. zeae*.

Following analysis of variance of Wr+Vr and Wr-Vr values, dominance and epistasis was not detected for plant height, number of root lesions, *Meloidogyne* spp. and grain yield since non-significant effects were obtained. Therefore, the observed heterosis can be attributed to overdominance genes detected in the Wr/Vr regression graphs. However, according to Hayman (1957), epistasis is likely to be detected whenever the heritability is high and a large number of families is compared. Related studies detected non-allelic interaction for *Meloidogyne incognita* resistance on upland cotton following analysis of variance on Wr–Vr

(Zhang *et al.*, 2007). The same authors found significant variance for Wr + Vr indicating the existence of dominance effects for *M. incognita* resistance on upland cotton. For *P. zeae*, mean squares for Wr-Vr had a significant effect, which confirms presence of epistasis in the inheritance of *P. zeae* resistance.

4.5 Conclusions

The following conclusions were drawn from the study:

- General combining ability contributed the most in reduction of *P. zeae* and *Meloidogyne* densities, and increase in root mass. Inbreds MP709 and CML206 had the best GCA effects for *P. zeae* resistance. Inbreds CML444, CML312 and CML395 enhanced grain yield with positive GCA effects.
- Specific combining ability was important in enhancing plant height and grain yield. Hybrids which had the highest SCA effects for grain yield under nematode infestation were MP709/CML312, 5057/CML395, 5057/CML312, CML206/CML312, CML444/CML312 and CML395/CML312.
- Poor adaptation of the inbred MP709 to tropical conditions compromised yield leading to negative reciprocal effects in hybrids MP709/CML444 and MP709/CML395 as verified from the positive maternal effects observed under nematode infestation. Hybrid MP709/CML312 displayed positive significant non-maternal effects for grain yield.
- The analysis for genetic effects indicated overdominance to be influential in explaining the non-additive portion controlling inheritance for all traits examined. It was, however, more significant for plant height and grain yield. Parents with most of the dominant alleles for *P. zeae* resistance were MP709, 5057, CML206 and CML444. For high grain yield, parent CML444 contributed most of the dominant genes whereas MP709, CML395 and 5057 had similar frequency of both dominant and recessive genes. Average heterosis was in the desired direction for plant height, grain yield, *P. zeae* densities and number of root lesions.
- Though overdominance was supreme for most traits analysed, results obtained apply to the population sampled. This is because Crow (2000)'s review article doubts the existence of overdominance; instead it is pseudo-overdominance due to linkage disequilibrium. It is further argued in the same article that additive and dominance effects are the major factors in population variability and heterosis, and that although overdominance and epistasis exist, neither has been shown to be important at the population level.

A preponderance of dominant genes would favour pedigree test, sib test, progeny test or various combinations among them, to improve grain yield under nematode infestation.

Inbreds MP709 and CML206 were the best sources of nematode resistance and should be used in combination with germplasm with good agronomic traits to improve resistance. There is a clear need to further study the inheritance of the most common race/species of *Meloidogyne* on maize in Uganda.

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Appendices

Appendix 4.1: Performance of the inbred line accessions under field conditions at IITA-Namulonge

Means squares for inbreds line accessions

Source	DF	Plant height (cm)	Root mass (g)	Root lesion no.	P. zeae/100g frm	Melo/100g frm	MSV severity	NLB severity	Borers damage	Grain yield (t ha^{-1})
Rep	1	0.44	0.01	11.5	1.59	0.08	1.78	0.69	0.44	0.13
Treat	1	0.33	0.81	0.36	0.05	0.21	2.78***	0.69	1.00	4.39
Rep*Treat	1	2.73	1.52	1.85	0.41	0.08	0.00	0.03	0.11	0.05
Genotype	8	10.9***	5.72**	4.47**	1.09*	0.08*	2.25**	5.0***	0.94***	2.19***
Treat*Genotype	8	1.00	2.01	0.18	0.19	0.02	1.65*	0.44	0.69***	0.41
Rep*Genotype(Treat)	16	0.791	1.21	0.92	0.42	0.03	0.58	0.30	0.09	0.29

Growth response and other characteristics of the inbred line accessions under nematode infested field conditions at IITA-Namulonge

Genotype	Plant height (cm)	Root mass (g)	Root lesion number	P. zeae/100g frm	Melo/100g frm	MSV severity	NLB severity	Borers damage	Grain yield (t ha^{-1})
5057	9.0 (71.5)	4.5 (11.0)	3.8 (4.7)	8.3(4079)	7.3(639)	1.8	3.8	1.0	2.2
9450	9.5 (81.6)	6.0 (28.3)	4.1 (8.0)	7.4(873)	7.0(55)	1.5	2.8	1.0	2.9
CML206	9.5 (80.8)	5.5 (20.7)	3.7 (3.8)	7.4(753)	7.0(50)	1.0	2.8	1.0	1.8
CML312	11.0 (111.6)	5.6 (21.4)	5.6 (21.6)	8.1(2323)	7.2(309)	2.5	1.0	1.5	2.3
CML395	11.1 (112.4)	5.0 (15.5)	4.9 (14.4)	8.1(3015)	7.2(349)	1.3	2.0	1.5	2.5
CML444	9.7 (85.3)	5.4 (21.0)	4.3 (8.8)	7.9(1677)	7.1(192)	1.0	1.8	1.3	2.6
MP709	8.4 (62.5)	4.5 (10.4)	4.7 (12.7)	7.2(402)	6.9(3.8)	2.0	2.0	1.3	1.5
MP710	9.4 (79.7)	4.5 (10.5)	4.6 (12.0)	8.6(7552)	7.2(357)	2.3	4.3	1.0	0.4
MP712	8.6 (65.5)	4.3 (8.4)	4.1 (7.1)	7.1(240)	7.0(96)	3.3	4.0	2.5	1.6
$LSD_{(0.05)}$	1.3	1.6	1.4	1.6	0.4	1.9	3.8	1.0	1.4
CV(%)	10.1	14.4	13.3						7.3

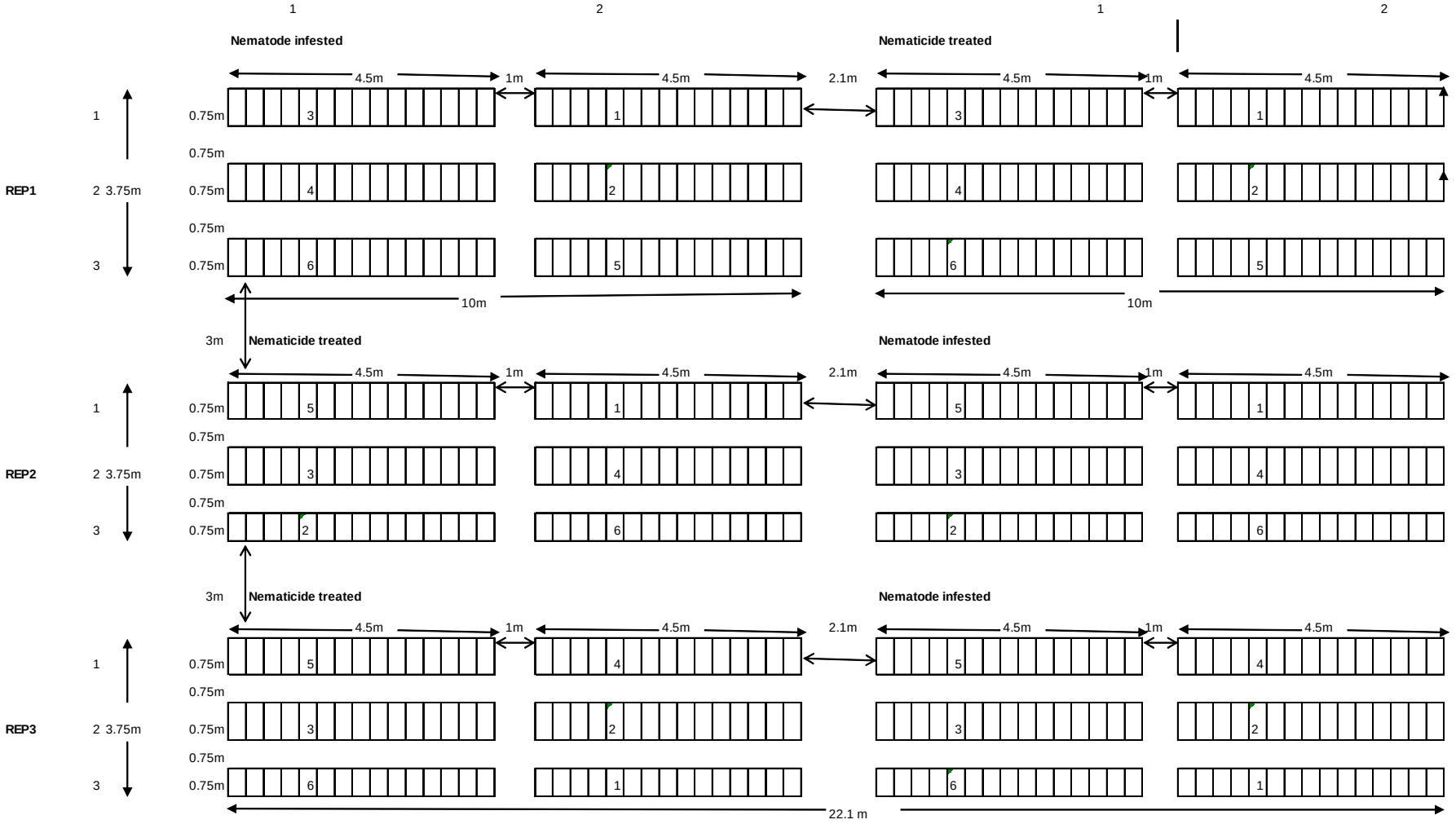
Rep = Replication, Treat = Treatment.
Values in parenthesis are untransformed means for data where transformation was done. Melo = *Meloidogyne* spp.

Appendix 4.2: Soil nutrient analysis results from the three experimental sites

Sites	**pH**	**OM**	**N**	**P**	Ca	Mg	K	Texture (%)		
		-------%-----		**ppm**	------- ($cmol.kg^{-1}$)------			Sand	Clay	Silt
Kabanyolo	4.9	4.9	0.25	12.0	1.51	0.68	0.21	41.4	46.3	12.7
Bufulubi	5.3	2.5	0.16	4.3	1.42	0.75	0.17	61.1	22.1	16.6
Namulonge	4.9	4.0	0.20	5.41	1.48	0.66	0.20	49.2	34.3	16.6
Critical values	5.2	3.0	0.20	5.0	0.4	0.1	0.2			
Sufficient levels	5.2-7.0	6.0	0.30	20.0	2.0	0.6	0.5			

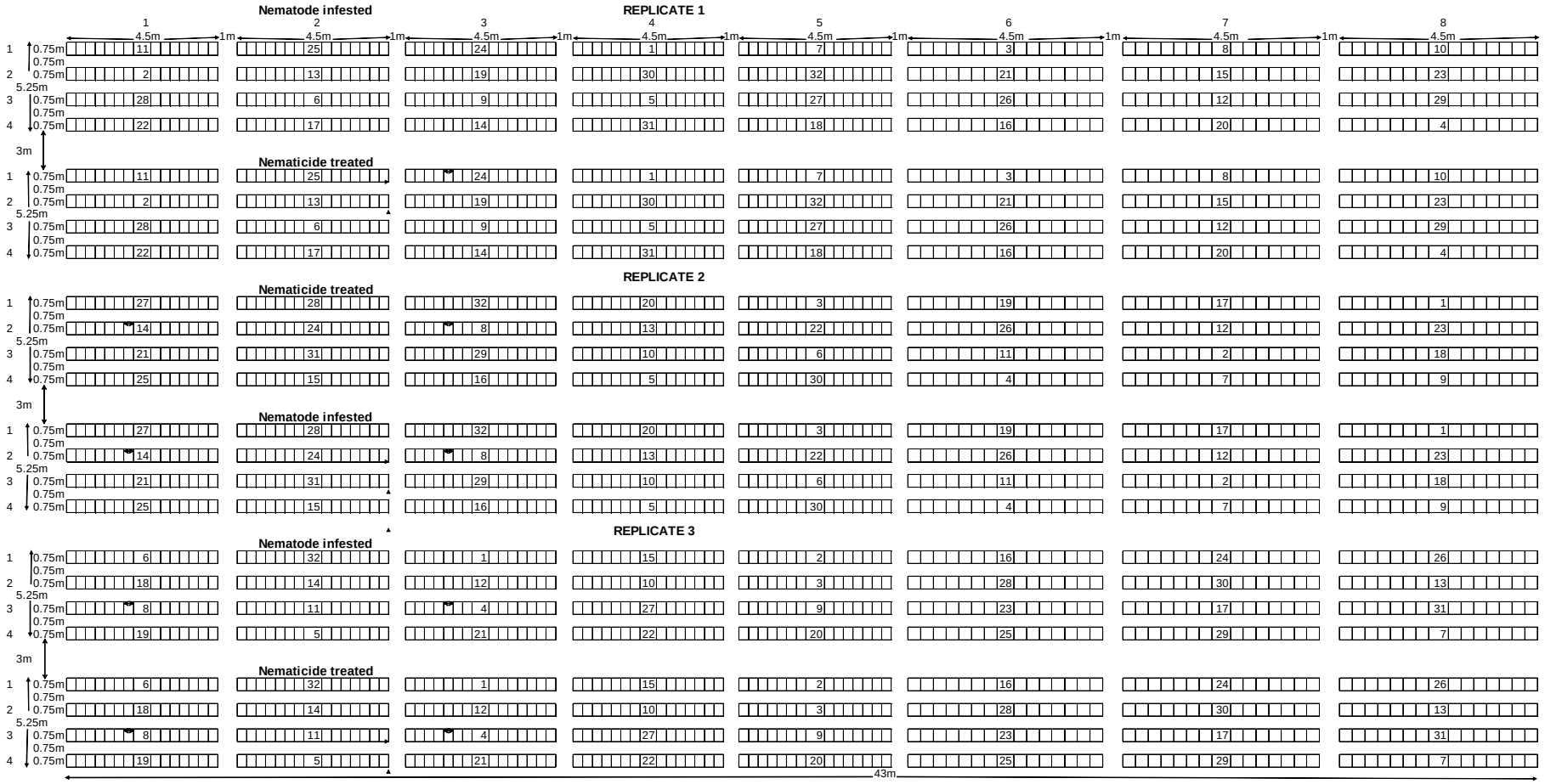
Results based on average of 10 soil samples collected from each site at 5-15 cm soil depth

Appendix 4.3: Field layout for experimental design of the multilocation evaluation of parents used in the diallel, arranged in a 3 x 2 alpha lattice in split plots



1) MP709, 2) 5057, 3) CML206, 4) CML444, 5) CML395, 6) CML312. Each T or L-junction represents a hill/plant

Appendix 4.4: Field layout for experimental design of the multilocation evaluation of hybrids, arranged in an 8 x 4 α-lattice design in split plots in the combining ability study



Each T or L-junction represents a hill/plant

CHAPTER FIVE

Grain yield and heterosis of maize hybrids under nematode infested and nematicide treated conditions

Abstract

Nematodes are present on maize in Uganda though resistant genotypes have not been identified, and yield losses associated with their damage have not been quantified. The objective of this study was to determine the level of nematode resistance in 30 F_1 hybrids, and to estimate grain yield, heterosis and yield losses associated with maize hybrids under nematode infestation. The 30 F_1 hybrids and two local checks were evaluated in a split plot design with nematode treatment as the whole plot factor, and the hybrids as subplot factors arranged in an 8 x 4 α-lattice design. The experiment was replicated three times at three sites. The hybrids were also evaluated in a split plot design under screenhouse conditions at IITA-Namulonge. Screenhouse studies revealed 24 *Pratylenchus zeae* susceptible hybrids compared to only six *P. zeae* resistant hybrids. Grain yield across sites was higher by about 400 kg ha^{-1} under nematicide treatment than under nematode infestation. The nematode resistant hybrids exhibited high yields ranging from 5.0 to 8.4 t ha^{-1} compared to 5.0 t ha^{-1} obtained from the best check. Grain yield loss ranged between 1 and 28% among susceptible hybrids, indicating that nematodes can cause economic yield losses when susceptible cultivars are grown. Under field conditions, favourable heterosis was recorded on 18 hybrids for *P. zeae* densities, and only on three hybrids for *Meloidogyne* spp. densities. Under nematode infestation, only 16 hybrids had higher relative yield compared to the mean of both checks, the best check and the trial mean, whereas it was 20 hybrids under nematicide treatment. Hybrids CML312/CML206, CML444/CML395, CML395/CML444, CML444/CML312, CML312/CML444, CML395/CML312, CML312/CML395, CML312/5057, CML395/5057, 5057/CML444, 5057/CML206, CML395/MP709, CML444/MP709 had higher relative yield compared to the mean of both checks, the best check and the trial mean, both under nematode infestation and nematicide treatment, indicating stability of performance between stressed and non-stressed environment. Overall, hybrids with outstanding performance under nematode infestation were CML395/MP709, CML312/5057, CML312/CML206, CML312/CML444, CML395/CML312 and CML312/CML395. Five of the six hybrids had CML312 as a parent, while CML444 and CML395 were parents in two of the six hybrids each. Resistant hybrids can play an important role in the agronomic management of nematodes for greater productivity in maize.

Key words: Heterosis, Maize hybrids, *Meloidogyne* spp., *Pratylenchus zeae*, Relative yield, Yield losses.

5.1 Introduction

Maize is the most important cereal crop in sub-Saharan Africa, and the second most important food crop after cassava on the African continent (DeVries and Toenniessen, 2001; FAOSTAT, 2009). In sub-Saharan Africa, maize is mostly grown by small-scale farmers, who lack inputs such as fertilizer, chemicals, improved seed, irrigation, and labour (Infonet-Biovision, 2009). As a result, the yields hardly exceed 1.8 t ha^{-1} (FAOSTAT, 2009). The average grain yield of maize in Uganda among small-scale farmers is 1.4 t ha^{-1} (FAOSTAT, 2009), which is much lower than the yields as high as 8 t ha^{-1} under research conditions (NARO, 2002). Pests and diseases have been listed as the most important constraint to maize production among small-scale farmers in East and Southern Uganda (Kagoda *et al.*, 2010a). Nonetheless, some cultivars exist which offer resistance to most pests and diseases in Uganda, in addition to being tolerant to abiotic stress (Kikafunda *et al.*, 2001; Pratt *et al.*, 2003; Imanywoha *et al.*, 2005). The challenge of plant-parasitic nematodes has, however, not been addressed for maize, not only in Uganda but also in many African countries. The majority of districts in Uganda where maize is grown have sandy loam soils (Foster, 1982), which are the most favourable for nematodes (Norton *et al.*, 1978).

Across the globe, over 60 nematode species have been associated with maize (Jones and Perry, 2004; McDonald and Nicol, 2005). In Uganda, the nematodes *Pratylenchus zeae* and *Meloidogyne* spp. are the most serious root pests of maize (Butseya *et al.*, 2005; Talwana *et al.*, 2008; Kagoda *et al.*, 2010a), and have potential to cause economic yield losses. Several researchers have reported yield losses due to these nematodes. In Kenya, Bridge (1994) estimated a 50% yield loss due to root-lesion nematodes. A 12% yield loss was reported in South Africa due to *Meloidogyne* spp. (Keetch, 1989). In Nigeria, Egunjobi (1974) reported maize yield loss of 28.5% due to *Pratylenchus* spp. In Pakistan, a yield loss of 23% was reported in maize planted in soils highly infested with *P. zeae* and *Helicotylenchus indicus* (Khan *et al.*, 2009). Though nematode control options like the use of nematicides, crop rotation, and bare fallow are effective, they are inappropriate on a low value crop like maize (Sikora, 1992). However, host plant resistance as a nematode control option is cheap, safe, and poses no technical difficulties to the farmer, provided that resistance genes are readily available (Trudgill, 1991). For instance, mass screenings of hybrids and inbred lines have led to the identification of maize germplasm that is resistant to *P. zeae* (Kimenju *et al.*, 1998; Oyekanmi *et al.*, 2007) and *Meloidogyne* spp. (Windham and Williams, 1987; Windham and Williams, 1988; Windham and Williams, 1994a). Such nematode resistant germplasm, together with the local materials in a country like Uganda can be used effectively in developing nematode resistant/tolerant maize hybrids for specific agroecosystems. The objectives of this study were, therefore, to: i) determine level of nematode resistance in

maize hybrids; ii) estimate heterosis for nematode resistance and grain yield in maize; and iii) estimate grain yield loss associated with nematode infestation in maize. It was hypothesized that: i) variations in nematode resistance exist among maize hybrids; ii) hybrid vigour for nematode resistance and grain yield can be obtained among nematode resistant maize hybrids; and iii) plant-parasitic nematodes, if left unchallenged, can cause significant grain yield loss in susceptible maize hybrids.

5.2 Materials and methods

5.2.1 Study area

The study was conducted at three sites in Uganda, namely Namulonge (1 200 m.a.s.l; 0°32'N, 32°34'E), Bufulubi (1 130 m.a.s.l; 00° 49' N, 033° 42' E) and Kabanyolo (1 150 m.a.s.l; 0° 28'N, 32° 37'E), which are characterized by greater than 40% sandy soils (Chapter 4, Appendix 4.2).

5.2.2 Germplasm

A total of 30 F_1 hybrids (including reciprocals) from a 6 x 6 full diallel mating design conducted at the International Institute of Tropical Agriculture (IITA) – Namulonge farm were used. The hybrids were developed from four CIMMYT inbred lines namely CML206, CML312, CML395 and CML444, known for their adaptability to tropical conditions in Uganda; one inbred line (MP709) from Mississippi, USA known for being resistant to *Meloidogyne* spp.; and an inbred line 5057 from IITA, Nigeria known for its resistance to *P. zeae* (Chapter 4, Table 4.1). A nematode susceptible (H614D) and resistant check (DK8031), which were both adapted to maize growing conditions in Uganda were included in the evaluation trials.

5.2.3 Experimental designs for screening of the hybrids for nematode resistance

5.2.3.1 Evaluation of the hybrids under field conditions

Field areas naturally infested with nematodes were used for the evaluation trials. This is because maize roots which remain in the soil after harvest, as well as weeds and the soil itself, are a reservoir for nematodes and their eggs during the dry season. This is an important source of infestation for the early season crop (Egunjobi and Bolaji, 1979). However, where nematode initial populations (P_i) were very low, nematode infected maize root pieces were applied per plant. At planting, soil samples were collected per plot and taken to the laboratory for nematode extraction and counting. Where nematode populations in the soil (P_i) were found low (< 500 *P. zeae* and < 100 *Meloidogyne* spp. per 100 g of soil)

in the nematode unprotected plots, nematode infested maize roots were chopped into small pieces and applied per plant in the affected block. For example, in each of the sites used at Namulonge and Kabanyolo, 500 *P. zeae* and 70 *Meloidogyne* spp. were applied per plant in form of chopped root pieces in order to raise the nematode population in the soil to 600. Nematode infested maize roots were collected from pots and from nematode infested on-station field trials for use as inocula. To estimate the number and species of nematodes, the composite sample of roots collected were chopped, mixed thoroughly and weighed. Ten sub-samples each weighing 5 g were then randomly picked from the composite sample and nematodes extracted from each following procedures outlined by Coyne *et al.* (2007). Nematode counts were then conducted per species under a stereomicroscope from each of the 10 sub-samples. It is from these sub-samples that the average number of nematodes per 5 g of root was obtained and later used to estimate the mass of root pieces containing the required number of nematodes for inoculation per plant.

The genotypes for evaluation in the different sites constituted the 30 F_1 hybrids and the two local checks, DK8031 and H614D. The hybrids were evaluated in a split plot design with nematode treatments (nematode infested verses nematicide treatment) as whole plots and the hybrids as subplots with two replications at Namulonge and three replications at Kabanyolo and Bufulubi (Chapter 4, Appendix 4.4). The hybrids were arranged in an 8 x 4 spatially adjusted α-lattice design for each of the nematode treatments. Field inter and intra-row spacing was maintained at 75 cm x 30 cm. Two row plots were planted per genotype consisting of 16 plants per row. Two seeds were sown per hill and later thinned to one plant. Other standard agronomic practices such as hand weeding were implemented at all the sites.

Prior to planting, soil samples were taken from a depth of 15 cm from each demarcated plot for nematode counts. Fertilizer to boost growth was applied at planting at a rate of 7.5 kg N ha^{-1} and 19.2 kg P_2O_5 ha^{-1}, all in the form of Di-ammonium phosphate (DAP). Fenamiphos (Nemacur™), a non-volatile nematicide was applied at a rate of 2.5 kg ha^{-1} (≈ 2.3 g per plant) and incorporated 5 to 8 cm soil depth with a hand hoe prior to planting in the nematode protected plots (Rhoades, 1979; Taylor *et al.*, 1999). However, the nematicide treated plots were not entirely free from nematodes but had significantly reduced nematode densities.

5.2.3.2 Evaluation of the hybrids under screenhouse conditions

A screenhouse trial was conducted to assess resistance to *P. zeae* among the hybrids. The design of the trial was split plot with two replications (Appendix 5.1). Whole plot factors were the nematode treatments and the sub-plot factors were the 32 hybrids. Two maize seeds were sown in plastic pots of 15 cm diameter containing 2 500 ml of a potting mixture of heat-sterilized sandy loam soil and river sand (2:1). For each F_1 hybrid, 12 pots were prepared. The pots were placed on metallic mesh tables about 1 m from the ground to avoid contamination. Watering of the pots was done twice a week with 0.5 l of water each time. After 10 days of seedling growth, they were thinned to one seedling per pot and inoculated with 5 000 *P. zeae* mixed stages (Fig. 5.1).

Figure 5.1: (a) *Pratylenchus zeae* inoculum under a stereomicroscope (x40) (b) Inoculation of a potted maize seedling with *P. zeae* using a pipette. (Images by Frank Kagoda and Perez Muchunguzi, respectively).

5.2.4 *Pratylenchus zeae* and *Meloidogyne* spp. inoculum preparation

Pratylenchus zeae used for inoculation were initially extracted using a modified Baermann sieve method (Coyne *et al*., 2007) from infected maize roots, obtained from farmers' fields in Iganga District, Uganda. The *P. zeae* were multiplied on carrots (*Daucus carota* L.), cv. Nantes in the laboratory (Kagoda *et al.*, 2010b)). The *P. zeae* culture was also maintained on susceptible maize hybrid H614D in pots in a shadehouse at IITA-Namulonge farm. For *Meloidogyne* spp., galled tomato roots were used for egg production (Hussey and Barker, 1973) and generation of juveniles for inoculation. These were also maintained in pots under a shadehouse.

5.2.5 Quantification of nematode densities and assessment of root damage

For the field experiments, soil samples per plot were collected for nematode (vermiform) population counting by species at or shortly before planting (P_i). The soil samples were collected in each plot using a trowel to a depth of 15 cm, discarding the upper 5 cm (Todd and Oakley, 1996; Coyne *et al.*, 2006). About 10 soil sub-samples per plot were combined to

form one sample. From 50% flowering, root samples were taken from the root system of 10 randomly selected plants in each plot for final nematode (P_f) assessment in the field. In the screenhouse, all plants were uprooted starting at flowering stage for nematode assessment. At this stage, the nematodes were expected to have completed two generations (Taylor and Sasser, 1978; Windham and Williams, 1987; Dropkin, 1989).

In the laboratory, nematodes were extracted from a 100 ml soil sub-sample (P_i), and from a macerated 5 g fresh root mass (frm) sub-sample (P_f), using a modified Baermann sieve method (Coyne *et al.*, 2007). The samples were examined after a 48 hour extraction period, and nematodes counted using a stereomicroscope. Both P_i and P_f were estimated from three x 2 ml aliquots, taken from a 25 ml suspension. Therefore, $P_f - P_i$ refers to the nematode populations found in the roots after subtracting the initial population in the soil in that plot at time of planting (Forrest and Holliday, 1979). In the screenhouse, Oostenbrink's (1966) reproduction factor ($RF = P_f/P_i$) was used to assess resistance to nematodes with $RF \leq 1.5$ indicating resistance to nematodes, $1.5<RF\leq2.0$ indicating moderately resistant, $10\geq RF>2.0$ indicating susceptibility and $RF>10$ indicating very susceptible to nematodes (Ferris *et al.*, 1993), but with slight modifications. The RF was not estimated for the field nematodes because of the variations in P_i in the field plots, as a result of many biotic factors.

5.2.6 Assessment of yield and other agronomic traits

In the field, plant height was recorded at flowering stage. Plant height was measured as height from the base of the plant to the insertion of the first tassel branch of the same plant (Magorokosho *et al.*, 2007). Before harvest, number of stands was taken to enable in prolificacy assessment and calculation of grain yield. Grain yield was taken on an entire plot basis at harvest and later adjusted to 12.5% moisture (CIMMYT, 1985) using the formula:

$$\text{Grain Yield (t ha}^{-1}) = \frac{\text{Grain mass (kg/plot)} \times 10 \times (100 - \text{Grain moisture content})}{87.5 \diagup \text{Plot area}}$$

Relative differences in performance of plants among treatments was calculated as:

$$\frac{\text{Performance in nematicide treated plot - Performance in nematode infested plot}}{\text{Performance in nematode infested plot}} \times 100$$

A nematode resistance index (RI) for each genotype was calculated by comparing yield from nematode infested plots with yield from nematicide treated plots using the formula (Johnson, 1975): $\text{Resistance index} = \frac{\text{Yield of plants in nematode infested plot}}{\text{Yield of plants in nematicide treated plot}} \times 100$

Therefore, the larger the RI value, the greater the resistance of the genotype to parasitic nematodes (Johnson, 1975). Specifically, RI < 100 represents nematode susceptible hybrid

hence grain yield reduced by nematodes; RI = 100 represents nematode tolerant hybrid hence yield not affected by presence of nematodes; RI > 100 represents nematode resistant hybrid hence grain yield increased since nematode presence was suppressed.

Yield loss was calculated using the formula:

$$\text{Yield loss (\%)} = \frac{\text{Yield in nematicide treated plot - Yield in nematode infested plot}}{\text{Yield in nematicide treated plot}} \text{x100}$$

In the screenhouse, growth parameter assessment was similar to that described for the field experiments. Root damage was assessed from fresh root mass (frm) and the number of root lesions (necrotic roots) (Fig. 5.2).

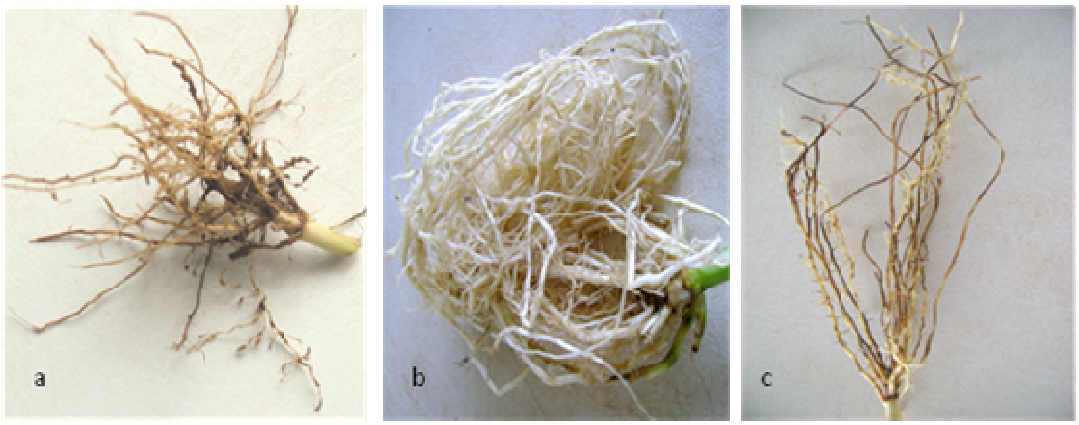


Figure 5.2: Roots from screenhouse trials that are: (a) Galled, (b) Clean, and (c) Necrotic.

5.2.7 Statistical analysis

Data from the field and screenhouse trials were subjected to analysis of variance as split plot experiments using the General Linear Model (Proc GLM) in SAS statistical package to enable separation of the variance components (Steel and Torrie, 1980). Data were tested for normality using the Proc Univariate normal plot procedure in SAS statistical package. Log and square root transformations were used where appropriate to transform the data prior to analysis. The nematode densities were $\log(x+10^3)$ transformed whereas grain yield was sqrt(x) transformed. Differences between means were compared using Tukey's studentized range test (Honestly Significant Difference) at P = 0.05 significant levels.

The following model was used for analysing data from the field experiments:

$Y = \mu + S + R + T + RT + H + HT + HR(T) + SR + ST + RTS + HS + THS + SHR(T)$

Where;

Y = observed value;

μ = Overall mean;

S = Site effect with 3 levels, i.e., Namulonge, Bufulubi and Kabanyolo.

R = Replication effect with 3 levels;

T = Treatment effect with 2 levels, i.e., nematodes infested and nematicide treatment;
H = Hybrid effect with 32 levels;
The error terms were five namely:
Error A = RT = Replication interaction with treatment effect - used to test significance of treatments;
Error B = HR(T) = Hybrid interaction with replication effects nested in treatment – used to test significance of hybrids, and treatment x hybrids interaction;
Error C = SR = Site interaction with replication effect – used to test significance of sites;
Error D = RTS = Replication x Treatment x Site interaction – used to test significance of sites x treatment interaction;
Error E = SHR(T) = Site x Hybrid x Replication nested in treatment interaction – used to test significance of sites x hybrid interaction, and sites x hybrid x treatment interaction.
For screenhouse data, the following model was used:
Y = μ + Rep + Treat + Rep x Treat + Hybrid + Hybrid x Treat + Rep x Hybrid (Treat)
Where;
Y = observed value;
μ = Overall mean;
Rep = Replication effect with 2 levels;
Treat = Treatment effect with 2 levels, i.e., nematodes infested and nematicide treatment;
H = Hybrid effect with 32 levels;
The error terms were two namely:
Error A = Rep x Treat = Replication interaction with treatment effect - used to test significance of treatments;
Error B = Rep x Hybrid (Treat) = Replication interaction with hybrids nested in treatment – used to test significance of hybrids, and treatment x hybrids interaction;

Pearson correlation and regression analyses were run using Proc corr and Proc reg procedures in SAS, respectively, to determine the type of relationships among traits.

Mid-parent heterosis (MPH) for nematode resistance and yield were calculated as the performance of the F_1 compared with the average performance of its parents (Falconer, 1981; Srivastava, 1991) as follows: $\frac{F_1 - MP}{MP} \times 100$, where F_1 = Mean of the F_1 hybrid performance, MP = mean of the two parents in the cross, i.e., $(P_1 + P_2)/2$, where P_1 and P_2 are the means of the inbred parents. To ascertain if there are differences in vigour between pairs of reciprocal hybrids, a t-test of significance was carried out on mid-parent heterosis

values obtained per replicate for *P. zeae, Meloidogyne* spp. and grain yield. The hypothesized mean difference between reciprocals was zero.

Relative yield (standard heterosis) was also calculated using the following formula:

$$\frac{\text{Yield of experimental hybrid}}{\text{Mean yield of the checks or Yield of the best check or Yield of trial mean}} \times 100$$

Ranking of hybrids based on grain yield was performed in Microsoft excel using the sort & filter procedure. Spearman rank correlation was then run using Proc corr procedure in SAS software to determine the differences between ranks of hybrids under nematode infestation and nematicide treatment.

5.3 Results

5.3.2 Performance of maize hybrids under nematode infested and nematicide treated conditions in the field

Variation among hybrids

Site effects had significant (P ≤ 0.05) variations for plant height, root mass and grain yield (Table 5.1).

Table 5.1: Mean squares for grain yield and other traits following evaluation of the 32 maize hybrids over three sites in Uganda

Source of variation	DF	Plant height(cm)	Root mass (g)	No. of root lesions	*P. zeae* (per 100g frm)	*Meloidogyne* spp. (per 100g frm)	Grain yield (t ha^{-1})
Site	2	1433*	246*	191	44.71	1.60	8.76**
Rep	2	48.2	31.9	11.7	2.01	0.23	1.02
Trt	1	73.7	9.5	23.4	245.3*	10.2*	1.33
Rep*Trt (Error A)	2	46.5	18.0	2.51	10.29	0.36	1.59
Hybrid	31	7.4***	2.5***	0.28	1.33***	0.12***	0.60***
Trt*Hybrid	31	3.0	0.6	0.16	0.39	0.05	0.14
Rep*Hybrid(Trt) (Error B)	124	3.2	1.0	0.29	0.38	0.04	0.16
Site*Rep (Error C)	3	35.4	3.7	39.1	21.01	2.16	0.21
Site*Trt	2	34.6	4.4	8.60	1.51	0.14	0.36
Site*Rep*Trt (Error D)	3	26.0	6.6	2.52	11.22	2.88	1.54
Site*Hybrid	62	3.2	0.9	0.30*	0.47	0.05	0.31***
Site*Trt*Hybrid	62	3.3	0.5	0.24	0.38	0.04	0.12
Site*Rep*Hybrid(Trt) (Error E)	186	3.9	0.8	0.21	0.37	0.05	0.12

Rep = Replicate; Trt = Treatment; *, **, & *** mean significance at P ≤ 0.05, P ≤ 0.01 and P ≤ 0.001, respectively.

Nematode treatments (Nematode infested vs Nematicide treated conditions) were significantly (P ≤ 0.05) different for only *P. zeae* and *Meloidogyne* spp. densities. The hybrids (including reciprocals) were significantly (P ≤ 0.05) different for all traits measured except number of root lesions. Site x Hybrid interactions were significantly (P ≤ 0.05) different for

number of root lesions and grain yield. Site x Treatment, Treatment x Hybrid and Site x Treatment x Hybrid interactions were not significantly different for any trait.

5.3.2.2 Performance of the hybrids across sites and treatments in the field

Plant height among the hybrids was significantly (P = 0.05) higher in five hybrids but lowest in four hybrids including the resistant check (Table 5.2). Four hybrids had the highest root mass, whereas five hybrids including both checks displayed the lowest root mass. The *P. zeae* densities were significantly (P = 0.05) lower in five hybrids, including the resistant check, but highest in eight hybrids. Grain yield was significantly (P = 0.05) higher (7.0-8.4 t ha^{-1}) in hybrids CML444/MP709, CML395/5057, CML312/CML206, CML444/CML395, CML444/CML312, CML312/CML444, CML395/CML312 and CML312/CML395 compared to only 4.7 to 5.0 t ha^{-1} obtained in MP709/5057, 5057/MP709, MP709/CML206, CML206/5057 and DK8031.

Table 5.2: Mean performance of individual F_1 hybrids across sites and nematode treatments

	Entry	Plant height	Root mass	No. of root lesions	*P. zeae* (per 100 g frm)	†Melo (per 100 g frm)	Grain yield (t ha^{-1})
1	MP709/5057	12.6(155)	6.2(30.0)	14.4	8.2(6329)	354	2.0(4.7)
	5057/MP709	12.7(156)	5.6(22.0)	13.7	8.1(5651)	251	2.2(5.0)
2	MP709/CML206	13.9(211)	6.8(37.3)	12.6	8.1(4478)	281	2.1(4.7)
	CML206/MP709	13.7(195)	6.6(36.8)	15.0	8.2(5148)	164	2.2(5.2)
3	MP709/CML444	13.1(167)	6.2(31.3)	15.2	8.5(8391)	418	2.3(5.8)
	CML444/MP709	14.3(203)	6.7(36.4)	14.4	8.2(6036)	573	2.6(7.0)
4	MP709/CML395	14.5(228)	6.6(35.7)	12.3	8.0(6450)	294	2.2(5.5)
	CML395/MP709	13.8(187)	6.1(29.4)	15.7	7.9(3821)	169	2.5(6.9)
5	MP709/CML312	14.7(239)	7.2(45.1)	13.5	8.3(5592)	372	2.4(6.3)
	CML312/MP709	14.9(245)	6.6(35.6)	14.4	8.5(9136)	605	2.3(6.0)
6	5057/CML206	12.3(145)	6.3(31.3)	11.8	8.3(5075)	547	2.4(6.0)
	CML206/5057	12.3(146)	6.5(34.4)	15.0	8.3(7599)	302	2.1(4.8)
7	5057/CML444	13.0(162)	6.7(36.5)	15.0	8.8(12548)	500	2.5(6.6)
	CML444/5057	12.8(159)	6.8(37.7)	14.9	8.7(8929)	557	2.4(6.2)
8	5057/CML395	12.7(155)	6.4(31.8)	15.1	8.6(9637)	328	2.4(6.1)
	CML395/5057	12.9(159)	6.4(32.7)	15.6	8.8(12619)	245	2.6(7.3)
9	5057/CML312	13.0(166)	7.1(42.8)	14.6	9.0(14222)	334	2.4(6.4)
	CML312/5057	13.3(171)	7.1(41.3)	15.5	8.6(9406)	411	2.5(6.6)
10	CML206/CML444	12.7(156)	7.3(45.7)	12.6	8.2(5341)	301	2.5(6.8)
	CML444/CML206	12.7(156)	7.4(47.1)	14.9	8.3(5207)	209	2.4(6.1)
11	CML206/CML395	12.1(141)	6.7(37.9)	16.2	8.0(4088)	334	2.2(5.9)
	CML395/CML206	12.7(161)	6.5(32.7)	17.9	8.2(4565)	110	2.4(6.1)
12	CML206/CML312	12.7(159)	7.4(46.7)	14.7	8.4(7016)	410	2.3(6.0)
	CML312/CML206	13.5(190)	7.1(41.7)	15.1	8.4(6676)	300	2.6(7.2)
13	CML444/CML395	13.0(165)	7.1(42.4)	15.7	8.3(5917)	1024	2.6(7.1)
	CML395/CML444	12.7(157)	6.9(40.2)	15.7	8.8(13049)	443	2.5(6.8)
14	CML444/CML312	13.7(185)	7.0(41.8)	16.4	8.7(12020)	495	2.7(7.6)
	CML312/CML444	14.5(219)	7.1(42.6)	18.5	8.7(11733)	263	2.7(7.8)
15	CML395/CML312	13.2(169)	6.9(39.8)	17.0	8.8(11830)	615	2.8(8.4)
	CML312/CML395	13.4(175)	6.4(33.0)	19.9	8.7(11579)	449	2.6(7.3)
	Checks						
16	DK8031	12.0(140)	6.1(28.9)	14.5	8.0(4840)	306	2.1(5.0)
17	H614D	14.1(195)	6.2(30.2)	16.3	8.5(8017)	647	2.1(5.4)
	$LSD_{(0.05)}$	**2.7**	**1.4**	**ns**	**1.0**	**ns**	**0.5**
	Mean	13.2(176)	6.7(36.8)	15.1	8.4(7905)	394	2.4(6.3)

Untransformed values are presented in parentheses for transformed traits. †*Meloidogyne* spp.

5.3.2.3 Mean performance of the hybrids under nematode treatments per site, associated yield loss and resistance index

At Bufulubi, plant height, number of root lesions and grain yield were not significantly different between nematode treatments though relatively higher plant height and grain yield were observed under nematicide treated plots than nematode infested plots (Table 5.3). Number of root lesions were also relatively higher under nematode infested plots than the nematicide treated plots. Root mass was significantly (P = 0.05) higher (12.5%) under nematicide treated plots than under nematode infestation. The *P. zeae* and *Meloidogyne* spp. densities were significantly (P = 0.05) higher under nematode infestation than under nematicide treated plots with relative differences of 73 and 63%, respectively. The resistance index and yield loss were 95.2 and 4.8%, respectively.

Table 5.3: Mean performance of hybrids, associated yield loss and resistance index under nematode infestation and nematicide treatment

	Plant height (cm)	Root mass (g)	No. of root lesions	*P. zeae* (per 100 g frm)	†Melo (per 100 g frm)	Grain yield (t ha^{-1})
Site: Bufulubi						
Nematodes	176.4	7.5(48)	25.0	9.6(18822)	7.4(670)	6.0
Nematicides	180.7	7.9(54)	23.4	8.3(5154)	7.1(249)	6.3
$LSD_{(0.05)}$	ns	0.3	ns	0.2	0.1	ns
Mean	178.6	7.7(51)	24.2	9.0(11988)	7.3(460)	6.2
Rel. Difference (%)	**2.4**	**12.5**	**-6.4**	**-73**	**-63**	**5.0**
Resistance index (%)						**95.2**
Yield loss (%)						**4.8**
Site: Kabanyolo						
Nematodes	14.5(201)	35.3	5.5(20.3)	8.8(9575)	7.2(387)	2.5(7.0)
Nematicides	16.3(275)	34.1	4.5(10.8)	7.2(464)	7.0(53)	2.6(7.4)
$LSD_{(0.05)}$	0.8	ns	0.2	0.2	0.1	0.1
Mean	15.4(238)	34.7	5.0(15.6)	8.0(5020)	7.1(220)	2.55(7.2)
Rel. Difference (%)	**37**	**-3.4**	**-47**	**-95**	**-86**	**5.7**
Resistance index (%)						**94.6**
Yield loss (%)						**5.4**
Site: Namulonge						
Nematodes	74.4	17.7	3.4(2.0)	8.8(10647)	7.4(983)	2.0(4.7)
Nematicides	81.0	19.9	3.2(0.2)	7.6(1567)	7.0(133)	2.2(5.2)
$LSD_{(0.05)}$	ns	ns	0.1	0.3	0.1	0.2
Mean	77.7	18.8	3.3(0.9)	8.2(6107)	7.2(558)	2.1(5.0)
Rel. Difference (%)	**9.0**	**12.4**	**-0.9**	**-85**	**-86**	**10.6**
Resistance index (%)						**90.4**
Yield loss (%)						**9.6**

Untransformed values are presented in parentheses for transformed traits. Relative differences and yield loss calculated based on untransformed values. †*Meloidogyne* spp.

At Kabanyolo, root mass was not significantly different between treatments (Table 5.3). Plant height was significantly higher (37%) under nematicide treated plots than nematode infested plots. Number of root lesions were significantly (P = 0.05) higher (47%) under nematode infested plots than the nematicide treated plots. The *P. zeae* and *Meloidogyne* spp. densities were significantly (P = 0.05) higher under nematode infestation than under nematicide treated plots with relative differences of 95 and 86%. Grain yield was significantly (P = 0.05) higher (5.7%) under nematicide treated plots compared to nematode infested plots. The resistance index and yield loss were 94.6 and 5.4%, respectively.

At Namulonge, plant height and root mass were not significant between treatments though relatively higher plant height and root mass were observed under nematicide treated plots than nematode infested plots (Table 5.3). Number of root lesions were significantly (P = 0.05) higher (0.9%) under nematode infested plots than the nematicide treated plots. The *P. zeae* and *Meloidogyne* spp. densities were significantly (P = 0.05) higher under nematode infestation than under nematicide treated plots with relative differences of 85% and 86%. Grain yield was significantly (P = 0.05) higher (11%) under nematicide treated plots compared to nematode infested plots. The resistance index and yield loss were 90.4% and 9.6%, respectively.

5.3.2.4 Yield losses and resistance index for each maize hybrid per site, and across sites

At Bufulubi, no significant differences in grain yield were recorded under nematicide treatment (Table 5.4). However, there were significant differences in yield among hybrids under nematode infestation. Yield loss ranged from 1 to 37% with highest loss observed in hybrids MP709/5057 and CML206/CML444. However, a total of 10 hybrids were resistant to nematodes (RI >100%) and recorded no grain yield losses at Bufulubi.

At Kabanyolo, grain yield was significantly higher than the mean of the trial in 14 hybrids and lowest in two hybrids (MP709/5057 and MP709/CML206) under nematode infestation ((Table 5.4). Most of the hybrids with high grain yield under nematode infestation similarly had high grain yield under nematicide treatment. The range of yield losses at Kabanyolo due to nematodes varied from 1 to 33% with the highest loss recorded in the hybrid MP709/5057. A total of six hybrids, including the resistant check, exhibited resistance to nematodes based on RI > 100% and absence of yield losses.

At Namulonge, no significant variation in grain yield was recorded under nematode infestation though hybrids 5057/CML444 and CML395/5057 had relatively higher grain yields

compared to MP709/CML395 and H614D (Table 5.4). Under nematicide treatment, the highest grain yield was recorded in CML312/CML444, CML206/CML444, CML395/5057, whereas the lowest grain yield was recorded in the hybrid check H614D and MP709/5057. Grain yield loss ranged from 1 to 66% with hybrid CML206/5057 having the highest loss. Highest resistance indices with no yield losses were recorded in 12 hybrids.

Across experimental sites, mean grain yield was higher under nematicide treated plots than nematode infested plots (Table 5.4). Among hybrids, grain yield was significantly (P = 0.05) higher in seven hybrids and lowest in three hybrids under nematode infestation. The same hybrids with higher grain yield under nematode infestation maintained high yields under nematicide treatment. Hybrids with the highest resistance index also had no grain yield loss under nematode infestation. The most resistant hybrids, based on the resistance index were CML206/CML395, CML206/MP709 and CML395/CML312, which had resistance indices ranging from 110 to 151. Both checks had resistance index below 100 and were therefore susceptible to nematodes. Tolerant (RI = 100%) hybrids were 5057/CML444 and 5057/CML395. Overall, yield loss ranged from 1 to 28% with hybrids CML206/CML444 and MP709/CML395 exhibiting the highest yield loss. The resistant check (DK8031) had a yield loss of 6% whereas the susceptible check (H614D) had a yield loss of 15%.

Table 5.4: Grain yield, resistance index (RI) and yield losses of individual hybrids and their reciprocals under nematodes and nematicide treated conditions

	Hybrids	Bufulubi				Kabanyolo				Namulonge				Across Locations			
		Yield (t ha^{-1})		†RI (%)	%Yld loss	Yield (t ha^{-1})		RI (%)	%Yld loss	Yield (t ha^{-1})		RI (%)	%Yld loss	Yield (t ha^{-1})		RI (%)	%Yld loss
		Nema	No			Nema	No			Nema	No			Nema	No		
1	MP709/5057	4.4	7.0	63	37	3.6	5.4	67	33	4.3	2.7	159	0	4.1	5.3	77	23
	5057/MP709	4.1	5.0	82	18	5.2	6.4	81	19	4.2	5.0	84	16	4.5	5.6	80	20
2	MP709/CML206	4.8	5.3	91	9	4.4	5.6	79	21	3.0	4.1	73	27	4.1	5.1	80	20
	CML206/MP709	4.8	4.4	109	0	6.5	5.8	112	0	4.5	4.3	105	0	5.4	4.9	110	0
3	MP709/CML444	6.6	7.3	90	10	5.8	7.4	78	22	6.6	7.7	86	14	5.1	6.5	78	22
	CML444/MP709	7.7	6.1	126	0	7.3	7.8	94	6	4.8	5.1	94	6	7.2	6.8	106	0
4	MP709/CML395	6.1	7.0	87	13	4.8	5.5	87	13	1.8	3.9	46	54	4.7	6.4	73	27
	CML395/MP709	6.0	7.2	83	17	8.3	8.3	100	0	6.3	6.2	102	0	6.7	7.1	94	6
5	MP709/CML312	6.4	7.4	86	14	5.9	7.0	84	16	2.4	6.5	37	63	5.9	6.6	89	11
	CML312/MP709	6.4	6.3	102	0	6.0	7.3	82	18	5.3	4.9	108	0	5.2	6.7	78	22
6	5057/CML206	5.4	4.8	113	0	6.5	6.1	107	0	5.2	4.9	106	0	6.1	5.8	105	0
	CML206/5057	3.8	4.9	78	22	4.6	5.6	82	18	2.2	6.5	34	66	4.4	5.2	85	15
7	5057/CML444	5.2	5.6	93	7	7.6	8.4	90	10	7.4	5.6	132	0	6.6	6.6	100	0
	CML444/5057	4.9	6.0	82	18	7.6	7.4	103	0	5.1	4.8	106	0	6.1	6.2	98	2
8	5057/CML395	5.7	5.8	98	2	6.8	7.1	96	4	5.5	4.9	112	0	6.1	6.1	100	0
	CML395/5057	7.5	7.0	107	0	7.2	6.7	107	0	7.2	8.8	82	18	7.3	7.4	99	1
9	5057/CML312	5.7	6.0	95	5	5.1	7.2	71	29	6.2	8.2	76	24	5.6	7.0	80	20
	CML312/5057	4.4	6.2	71	29	7.7	7.8	99	1	4.5	6.7	67	33	6.1	6.9	88	12
10	CML206/CML444	5.0	7.9	63	37	6.7	7.7	87	13	5.4	8.1	67	33	5.7	7.9	72	28
	CML444/CML206	6.6	7.4	89	11	6.3	7.1	89	11	3.9	4.0	98	3	5.7	6.4	89	11
11	CML206/CML395	6.2	4.3	144	0	11.1	5.8	191	0	2.6	3.7	70	30	7.1	4.7	151	0
	CML395/CML206	5.9	6.7	88	12	6.9	7.6	91	9	3.9	4.4	89	11	5.8	6.5	89	11
12	CML206/CML312	5.2	7.0	74	26	6.9	7.2	96	4	4.6	4.0	115	0	5.7	6.2	92	8
	CML312/CML206	6.5	7.0	93	7	8.5	9.3	91	9	5.0	5.3	94	6	6.9	7.4	93	7
13	CML444/CML395	6.4	5.6	114	0	8.5	9.0	94	6	6.3	5.9	107	0	7.2	6.9	104	0
	CML395/CML444	6.6	6.7	99	1	7.8	8.8	89	11	6.4	3.5	183	0	7.0	6.7	104	0
14	CML444/CML312	7.2	5.3	136	0	10.2	9.7	105	0	3.3	7.1	46	54	7.9	7.4	107	0
	CML312/CML444	7.5	6.5	115	0	9.4	9.5	99	1	5.3	8.2	65	35	7.6	8.0	95	5
15	CML395/CML312	11.2	7.0	160	0	8.3	10.2	81	19	5.9	4.1	144	0	8.8	8.0	110	0
	CML312/CML395	7.5	7.8	96	4	8.2	8.5	96	4	6.0	4.5	133	0	7.4	7.1	104	0
16	DK8031 (Check)	5.7	6.4	89	11	5.0	4.0	125	0	4.0	5.1	78	22	4.9	5.2	94	6
17	H614D (Check)	5.1	6.7	76	24	6.9	8.4	82	18	1.6	0.7	229	0	4.5	5.3	85	15
	$LSD_{(0.05)}$	1.4	ns			1.0	0.6			ns	2.2			0.8	ns		
	Mean	**6.0**	**6.3**			**6.9**	**7.4**			4.7	5.3			**6.0**	**6.4**		

†Resistance index, Nema = Nematode infested, No = Nematicide treated.

5.3.3 Pearson correlation between grain yield and other traits under field conditions

Under nematode infestation, grain yield was significant (P < 0.001) and positively correlated with plant height and root mass (Table 5.5a). However, grain yield had significant (P < 0.001) and negative correlation with number of root lesions, and non-significant (P > 0.05) and negative correlation with *P. zeae* and *Meloidogyne* spp. densities. Under nematicide treatment, grain yield had significant (P < 0.001) and positive correlation with plant height, root mass and number of root lesions, but had non-significant (P > 0.05) and negative correlation with *P. zeae* and *Meloidogyne* spp. densities.

Table 5.5a: Pearson correlation coefficients under nematode infestation (below diagonal) and nematicide treated (above diagonal) plots

	Plant height	Root mass	No. of root lesions	*P. zeae*	*Meloidogyne* spp.	Grain yield
Plant height (cm)		0.382***	0.386***	-0.077ns	-0.084ns	0.327***
Root mass (g)	0.571***		0.569***	0.481***	0.100ns	0.277***
Number of root Lesions	-0.679***	-0.385***		0.292***	0.275***	0.294***
P. zeae (per 100g frm)	-0.129*	-0.277***	0.039ns		0.329***	-0.028ns
Meloidogyne spp. (per 100g frm)	-0.120*	0.049ns	-0.223***	0.516***		-0.008ns
Grain yield (t ha^{-1})	0.464***	0.350***	-0.281***	-0.097ns	-0.029ns	

*, **, and *** represent significance at P ≤ 0.05, P ≤ 0.01 and P ≤ 0.001, respectively. ns = not significant.

Number of root lesions was significant (P < 0.05) and negatively correlated with plant height, root mass and *Meloidogyne* spp. densities under nematode infestation. However, under nematicide treatment, number of root lesions was significant (P < 0.001) and positively correlated with plant height, root mass and *Meloidogyne* spp. densities. *Pratylenchus zeae* densities were significant (P < 0.05) and negatively correlated with plant height and root mass under nematode infestation but had a significant and positive relationship with *Meloidogyne* spp. densities. Under nematicide treatment, *P. zeae* had a significant (P < 0.001) and positive correlation with root mass, number of root lesions and *Meloidogyne* spp. densities but had a negative relationship with plant height. *Meloidogyne* spp. densities had a significant (P < 0.05) and negative correlation with plant height and number of root lesions under nematode infestation.

5.3.4 Linear regression analysis

Plant height and root mass exhibited a positive and highly significant (P < 0.001) regression with grain yield under both nematode infested and nematicide treated plots (Table 5.5b). The number of root lesions, *P. zeae* densities and *Meloidogyne* spp. densities had negative and non-significant regression with grain yield.

Table 5.5b: Linear regression relationship between grain yield and other traits under nematodes and nematicide treatments

Variable	Regression equation	†R-square value (%)	P- value
Nematode infested conditions			
Plant height	Y =1.06+0.10x	21.6	<0.001
Root mass	Y =1.54+0.12x	12.3	<0.001
Number of root lesions	Y =1.74-0.12x	0.8	0.122
P. zeae densities	Y =1.91-0.05x	0.9	0.132
Meloidogyne spp. densities	Y =2.64-0.04x	0.1	0.648
Nematicide treated conditions			
Plant height	Y =1.89+0.04x	10.7	<0.001
Root mass	Y =1.79+0.09x	7.7	<0.001
Number of root lesions	Y =1.91-0.11x	0.6	0.461
P. zeae densities	Y =2.55-0.02x	0.1	0.665
Meloidogyne spp. densities	Y =2.59-0.02x	0.001	0.896

†R-square = coefficient of determination, Y = grain yield coefficient, x = corresponding variable.

5.3.5 Response of the hybrids to *P. zeae* infection under screenhouse conditions

Analysis of variance

Following *P. zeae* inoculation in the screenhouse, the hybrids exhibited significant differences in plant height (P = 0.01) and root mass (P = 0.002) (Table 5.6). The nematode treatments were not significantly different for plant height (P > 0.05) but were different for root mass (P = 0.05). Treatment x Hybrid interactions were not significant for plant height and root mass.

Number of root lesions, *P. zeae* densities and reproduction factor (RF) were not included in the ANOVA because of the zero values in the uninoculated pots but their means have been presented (Table 5.8).

Table 5.6: Analysis of variance for plant height and root mass under screenhouse conditions

Source of variation	DF	Plant height (cm)	Root mass (g)
Rep	1	1.382	187.32
Treat	1	0.006	20.35*
Rep xTreat (Error A)	1	19.50	0.125
Hybrid	31	69.2**	33.34**
Treat x Hybrid	31	22.4	7.63
Rep x Hybrid (Treat) (Error B)	62	36.1	13.9

5.3.5.1 Means for *P. zeae* densities, reproduction factor (RF) and other traits following *P. zeae* inoculation

Root mass was significantly higher in the uninoculated pots than in the *P. zeae* inoculated pots (Fig. 5.3). Mean *P. zeae* density was 4 4007 per 100 g frm in the inoculated pots whereas the uninoculated pots had no *P. zeae*. Mean RF in the inoculated pots was 8.8.

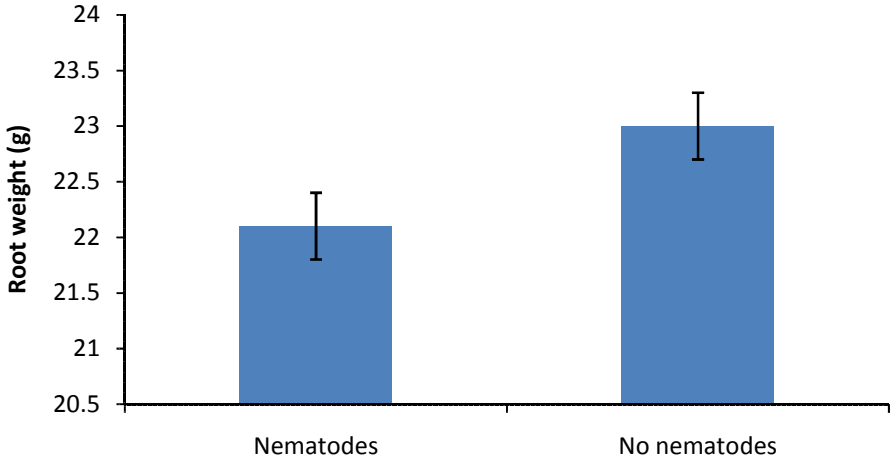


Figure 5.3: Mean root mass of maize hybrids under *P. zeae* inoculation and uninoculated screenhouse trial

Following *P. zeae* inoculation, plants were significantly taller (P = 0.05) in hybrids MP709/CML395, MP709/CML312, CML395/CML444, CML395/CML312 and the check H614D. Hybrids MP709/CML444, CML206/5057 and CML444/CML206 were the shortest (Table 5.7). Root mass was significantly (P < 0.05) higher in hybrids CML312/MP709, CML395/MP709 and 5057/MP709 but very low in hybrids CML444/CML206, MP709/CML206 and MP709/5057. Based on the RF, 10 hybrids (including the susceptible check) were very susceptible to *P. zeae*, whereas 15 hybrids were susceptible. Only five hybrids (including the resistant check) had resistance to *P. zeae*, whereas two hybrids were moderately resistant. Some of the hybrids which had the highest susceptibility to *P. zeae*

were 5057/CML206, CML444/CML312 and CML312/MP709. The most resistant hybrids were 5057/MP709, 5057/CML444, CML206/CML312, CML395/CML312.

Table 5.7: *P. zeae* densities, reproduction factor and *P. zeae* status of the hybrids in inoculated pots in the screenhouse

	Hybrids	Plant height (cm)	Root mass (g)	†P.zeae (per 100 g frm) (P_f)	††RF (P_f/P_i)	*P. zeae* status*
1	MP709/5057	41.1	19.5	56563	11.3	Very susceptible
	5057/MP709	44.1	26.6	4438	0.9	Resistant
2	MP709/CML206	44.1	16.3	20656	4.1	Susceptible
	CML206/MP709	42.5	21.5	31375	6.3	Susceptible
3	MP709/CML444	38.9	25.2	16063	3.2	Susceptible
	CML444/MP709	44.5	25.8	85219	17.0	Very susceptible
4	MP709/CML395	46.3	20.0	14469	2.9	Susceptible
	CML395/MP709	41.1	27.5	47781	9.6	Susceptible
5	MP709/CML312	47.7	22.4	9125	1.8	Moderately Resistant
	CML312/MP709	45.0	30.9	103375	20.7	Very susceptible
6	5057/CML206	40.4	20.0	188969	37.8	Very Susceptible
	CML206/5057	36.1	21.3	37625	7.5	Susceptible
7	5057/CML444	45.8	23.8	4531	0.9	Resistant
	CML444/5057	43.0	21.2	36156	7.2	Susceptible
8	5057/CML395	41.6	24.3	41125	8.2	Susceptible
	CML395/5057	42.8	22.4	67375	13.5	Very susceptible
9	5057/CML312	41.0	24.8	74906	15.0	Very susceptible
	CML312/5057	45.8	22.8	27969	5.6	Susceptible
10	CML206/CML444	42.0	20.6	27906	5.6	Susceptible
	CML444/CML206	34.6	19.2	34594	6.9	Susceptible
11	CML206/CML395	41.9	18.7	44563	8.9	Susceptible
	CML395/CML206	42.4	20.1	44219	8.8	Susceptible
12	CML206/CML312	45.6	23.3	2750	0.6	Resistant
	CML312/CML206	42.5	25.2	8563	1.7	Moderately Resistant
13	CML444/CML395	45.2	23.3	60719	12.1	Very susceptible
	CML395/CML444	50.2	20.3	21313	4.3	Susceptible
14	CML444/CML312	44.2	21.3	155750	31.2	Very susceptible
	CML312/CML444	43.8	22.2	54594	10.9	Very susceptible
15	CML395/CML312	49.1	21.2	3656	0.7	Resistant
	CML312/CML395	46.7	24.0	21094	4.2	Susceptible
	Checks					
16	DK8031	43.9	21.4	5781	1.2	Resistant
17	H614D	57.9	22.5	55000	11.0	Very susceptible
	Mean	43.8	22.5	44007	8.8	
	$LSD_{0.05}$	16.8	10.4			

†The P_i used to calculate reproductive factor (RF) is 5000 *P. zeae* (juveniles and adults) per pot. ††Values obtained only in the *P. zeae* inoculated pots. **P. zeae* resistant hybrids have RF ≤ 1.5 based on untransformed RF means.

Some hybrids maintained *P. zeae* resistance both in the screenhouse and in the field. Hybrids 5057/MP709 and 5057/CML444 were among the most resistant to *P. zeae* in the screenhouse. They performed relatively well in the field with grain yields of 5.0 and 6.6 t ha^{-1}, respectively, despite adaptation problems such as susceptibility to maize streak virus and northern leaf blight. Similarly, the hybrid CML395/CML312 exhibited *P. zeae* resistance in the screenhouse and yielded highly in the field with 8.4 t ha^{-1}. Hybrids MP709/CML312 and CML312/CML206 were relatively resistant to *P. zeae* in the screenhouse and in the field resulting in grain yields of 6.3 and 7.2 t ha^{-1}, respectively. Therefore, the hybrids

MP709/CML312 and CML312/CML206 outperformed the resistant check DK8031 (5.0 t ha^{-1}).

5.3.6 Relative yield and heterosis for nematode resistance and grain yield of the maize hybrids

Heterosis was computed for *P. zeae* under screenhouse and field conditions, and for *Meloidogyne* spp. and grain yield under field conditions. Negative heterosis for *P. zeae* and *Meloidogyne* spp. densities is an indication of an F_1 which is superior in resistance to nematodes compared to the mid-parent, susceptible parent or resistant parent. Contrary, positive heterosis for grain yield is an indication that the hybrids were superior in grain yield compared to the mid-parent, susceptible parent or resistant parent.

5.3.6.1 Heterosis for resistance to *P. zeae* under screenhouse conditions

A total of 14 hybrids displayed negative heterosis for *P. zeae* resistance, whereas 16 hybrids had positive heterosis (Table 5.8a). Differences between reciprocals were significant for MP709/5057 ($P < 0.01$) and CML444/CML312 ($P < 0.05$). No significant reciprocal differences were observed for the rest of the hybrids.

Table 5.8a: Heterosis for resistance to *P. zeae* under screenhouse conditions

	Hybrid	F_1	P_1	P_2	†MPH (%)
1	MP709/5057	56563	5563	91500	16.5
	5057/MP709	4438	91500	5563	-90.9
	t-value				11.0**
2	MP709/CML206	20656	5563	11521	141.8
	CML206/MP709	31375	11521	5563	267.3
	t-value				-0.18ns
3	MP709/CML444	16063	5563	39604	-28.9
	CML444/MP709	85219	39604	5563	277.4
	t-value				-0.95ns
4	MP709/CML395	14469	5563	31083	-21.0
	CML395xMP709	47781	31083	5563	160.8
	t-value				-0.752ns
5	MP709/CML312	9125	5563	35875	-56.0
	CML312/MP709	103375	35875	5563	398.9
	t-value				-0.957ns
6	5057xCML206	188969	91500	11521	266.9
	CML206x5057	37625	11521	91500	-27.0
	t-value				0.9ns
7	5057xCML444	4531	91500	39604	-93.1
	CML444x5057	36156	39604	91500	-44.8
	t-value				-1.07ns
8	5057/CML395	41125	91500	31083	-32.9
	CML395/5057	67375	31083	91500	9.9
	t-value				-0.468ns
9	5057xCML312	74906	91500	35875	17.6
	CML312x5057	27969	35875	91500	-56.1
	t-value				1.02ns
10	CML206/CML444	27906	11521	39604	9.2
	CML444/CML206	34594	39604	11521	35.3
	t-value				-0.1ns
11	CML206xCML395	44563	11521	31083	109.2
	CML395xCML206	44219	31083	11521	107.6
	t-value				-0.602ns
12	CML206xCML312	2750	11521	35875	-88.4
	CML312xCML206	8563	35875	11521	-63.9
	t-value				-1.388ns
13	CML444/CML395	60719	39604	31083	71.8
	CML395/CML444	21313	31083	39604	-39.7
	t-value				1.08ns
14	CML444/CML312	155750	39604	35875	312.7
	CML312/CML444	54594	35875	39604	44.7
	t-value				5.57*
15	CML395/CML312	3656	31083	35875	-89.1
	CML312/CML395	21094	35875	31083	-37.0
	t-value				-1.131ns

†Mid-parent heterosis presented as a percentage

5.3.6.2 Heterosis for resistance to *P. zeae*, *Meloidogyne* spp. and grain yield under field conditions

Negative heterosis for *P. zeae* was recorded on 18 hybrids, whereas 12 hybrids had positive heterosis (Table 5.8b). For *Meloidogyne* spp., negative heterosis was recorded on three hybrids, whereas 27 hybrids displayed positive heterosis. No significant reciprocal differences were observed for both *P. zeae* and *Meloidogyne* spp. densities in all the hybrids.

Table 5.8b: Mid-parent heterosis recorded for the F_1 hybrids and their reciprocals for resistance to *P. zeae*, and *Meloidogyne* spp. under field conditions

		P. zeae (per 100 g frm)		*Meloidogyne* spp. (per 100 g frm)	
	Hybrid	F_1	MPH (%)	F_1	MPH (%)
1	MP709/5057	11516	46	551	159
	5057/MP709	10820	37	427	100
	t-value		-0.025ns		-0.307ns
2	MP709/CML206	7460	-4	532	115
	CML206/MP709	9047	17	248	0
	t-value		0.016ns		1.69ns
3	MP709/CML444	14005	22	629	160
	CML444/MP709	10493	-8	944	290
	t-value		0.438ns		-1.74ns
4	MP709/CML395	12452	2	526	66
	CML395/MP709	7011	-43	255	-20
	t-value		0.88ns		0.57ns
5	MP709/CML312	9458	-44	583	39
	CML312/MP709	16886	-1	1138	171
	t-value		-0.436ns		-0.245ns
6	5057/CML206	8701	-5	605	168
	CML206/5057	12143	32	512	127
	t-value		-0.642ns		-0.02ns
7	5057/CML444	19676	52	807	265
	CML444/5057	14524	12	879	298
	t-value		0.728ns		0.173ns
8	5057/CML395	17319	27	485	64
	CML395/5057	21304	56	376	27
	t-value		0.049ns		0.33ns
9	5057/CML312	20791	13	581	45
	CML312/5057	16119	-13	737	84
	t-value		-0.029ns		-0.82ns
10	CML206/CML444	9041	-29	488	91
	CML444/CML206	8363	-35	306	20
	t-value		-0.161ns		0.596ns
11	CML206/CML395	7409	-45	518	57
	CML395/CML206	7631	-44	200	-39
	t-value		0.236ns		2.56ns
12	CML206/CML312	10987	-40	718	66
	CML312/CML206	10486	-43	475	10
	t-value		0.218ns		0.88ns
13	CML444/CML395	10126	-41	1866	473
	CML395/CML444	21075	22	667	105
	t-value		-0.977ns		-0.211ns
14	CML444/CML312	18162	-18	803	87
	CML312/CML444	20039	-9	347	-19
	t-value		0.486ns		1.76ns
15	CML395/CML312	20190	-11	1023	103
	CML312/CML395	20016	-12	773	53
	t-value		-0.103ns		0.78ns

All hybrids exhibited positive heterosis for grain yield under nematode infestation and nematicide treated plots in the field (Table 5.8c). Under nematode infestation, heterosis ranged from 189 to 485%, whereas a narrow range of 205 to 363% was recorded under nematicide treatment. Significant ($P < 0.05$) reciprocal differences were observed for hybrid MP709/CML395 under nematode infestation.

Table 5.8c: Mid-parent heterosis observed in F_1 hybrids and their reciprocal crosses for grain yield

	Hybrid	Nematodes infestation		Nematicide treatment	
		F_1	MPH (%)	F_1	MPH (%)
1	MP709/5057	4.1	253	5.3	249
	5057/MP709	4.5	288	5.6	268
	t-value		-1.203ns		-1.058ns
2	MP709/CML206	4.1	276	5.1	312
	CML206/MP709	5.4	395	4.9	298
	t-value		-2.29ns		-0.179ns
3	MP709/CML444	5.1	189	6.5	227
	CML444/MP709	7.2	308	6.8	243
	t-value		-2.15ns		-0.224ns
4	MP709/CML395	4.7	226	6.4	283
	CML395/MP709	6.7	365	7.1	320
	t-value		-3.81*		-0.927ns
5	MP709/CML312	5.9	372	6.6	239
	CML312/MP709	5.2	316	6.7	244
	t-value		-0.213ns		0.043ns
6	5057/CML206	6.1	460	5.8	363
	CML206/5057	4.4	304	5.2	318
	t-value		1.69ns		0.634ns
7	5057/CML444	6.6	274	6.6	234
	CML444/5057	6.1	246	6.2	213
	t-value		-0.097ns		0.325ns
8	5057/CML395	6.1	324	6.1	260
	CML395/5057	7.3	407	7.4	337
	t-value		-0.972ns		0.435ns
9	5057/CML312	5.6	348	7	260
	CML312/5057	6.1	388	6.9	255
	t-value		-1.892ns		0.064ns
10	CML206/CML444	5.7	236	7.9	363
	CML444/CML206	5.7	236	6.4	278
	t-value		0.781ns		0.662ns
11	CML206/CML395	7.1	418	4.7	233
	CML395/CML206	5.8	323	6.5	357
	t-value		0.787ns		-1.011ns
12	CML206/CML312	5.7	383	6.2	272
	CML312/CML206	6.9	485	7.4	344
	t-value		-0.985ns		-1.172ns
13	CML444/CML395	7.2	252	6.9	224
	CML395/CML444	7	242	6.7	212
	t-value		0.416ns		-0.078ns
14	CML444/CML312	7.9	326	7.4	205
	CML312/CML444	7.6	310	8	234
	t-value		0.839ns		-0.649ns
15	CML395/CML312	8.8	475	8	276
	CML312/CML395	7.4	384	7.1	238
	t-value		0.743ns		0.826ns

Results of standard heterosis for grain yield (relative yield) and ranking of the hybrids under nematode infestation and nematicide treated plots in the field are presented in Table 5.9. Under nematode infestation, 25 out of 30 hybrids outperformed the mean of both checks since they had their relative yield above 100%. Performance of hybrid MP709/CML395 was similar to the mean of the checks since its relative yield was 100%. Under nematode infestation, 26 out of 30 hybrids performed better than the best check (DK8031). However, only 16 hybrids out of 30 outperformed the trial mean under nematode infestation.

Table 5.9: Relative yield of hybrids (standard heterosis) and their rank order under nematode infestation and nematicide treated plots

		Nematodes infested plots					Nematicide treated plots				
				Relative yield over:					Relative yield over:		
	Hybrid	Grain yield	Rank	Mean of checks	Best check	Trial mean	Grain yield	Rank	Mean of checks	Best check	Trial mean
1	MP709/5057	4.1	30	87	84	68	5.3	26	101	100	83
	5057/MP709	4.5	27	96	92	75	5.6	25	107	106	88
2	MP709/CML206	4.1	29	87	84	68	5.1	28	97	96	80
	CML206/MP709	5.4	23	115	110	90	4.9	29	93	92	77
3	MP709/CML444	5.1	25	109	104	85	6.5	17	124	123	102
	CML444/MP709	7.2	6	153	147	120	6.8	12	130	128	106
4	MP709/CML395	4.7	26	100	96	78	6.4	20	122	121	100
	CML395/MP709	6.7	11	143	137	112	7.1	8	135	134	111
5	MP709/CML312	5.9	17	126	120	98	6.6	16	126	125	103
	CML312/MP709	5.2	24	111	106	87	6.7	13	128	126	105
6	5057/CML206	6.1	13	130	124	102	5.8	24	110	109	91
	CML206/5057	4.4	28	94	90	73	5.2	27	99	98	81
7	5057/CML444	6.6	12	140	135	110	6.6	15	126	125	103
	CML444/5057	6.1	14	130	124	102	6.2	22	118	117	97
8	5057/CML395	6.1	16	130	124	102	6.1	23	116	115	95
	CML395/5057	7.3	5	155	149	122	7.4	5	141	140	116
9	5057/CML312	5.6	22	119	114	93	7.0	9	133	132	109
	CML312/5057	6.1	15	130	124	102	6.9	11	131	130	108
10	CML206/CML444	5.7	20	121	116	95	7.9	3	150	149	123
	CML444/CML206	5.7	21	121	116	95	6.4	19	122	121	100
11	CML206/CML395	7.1	8	151	145	118	4.7	30	90	89	73
	CML395/CML206	5.8	18	123	118	97	6.5	18	124	123	102
12	CML206/CML312	5.7	19	121	116	95	6.2	21	118	117	97
	CML312/CML206	6.9	10	147	141	115	7.4	4	141	140	116
13	CML444/CML395	7.2	7	153	147	120	6.9	10	131	130	108
	CML395/CML444	7.0	9	149	143	117	6.7	14	128	126	105
14	CML444/CML312	7.9	2	168	161	132	7.4	6	141	140	116
	CML312/CML444	7.6	3	162	155	127	8.0	1	152	151	125
15	CML395/CML312	8.8	1	187	180	147	8.0	2	152	151	125
	CML312/CML395	7.4	4	157	151	123	7.1	7	135	134	111
16	DK8031	4.9				82	5.2				81
17	H614D	4.5				75	5.3				83
	Trial mean	6.0					6.4				
	Check Mean	4.7					5.25				

Under nematicide treatment, 26 hybrids out of 30 performed better than the mean of checks, whereas 25 hybrids outperformed the best check. A total of 18 hybrids performed better than the trial mean. One hybrid (MP709/5057) yielded as good as the best check, whereas two hybrids (MP709/CML395 and CML444/CML206), yielded as well as the trial mean. Hybrids CML312/CML206, CML444/CML395, CML395/CML444, CML444/CML312, CML312/CML444, CML395/CML312, CML312/CML395, CML312/5057, CML395/5057, 5057/CML444, 5057/CML206, CML395/MP709, CML444/MP709 had higher relative yield compared to the mean of both checks, the best check and the trial mean, both under nematode infestation and nematicide treatment, indicating stability of performance between stressed and non-stressed environment.

Spearman rank correlation showed a change in rank order in grain yield in most of the hybrids under nematode infestation when compared to nematicide treated plots (r = 0.636; P = 0.0002)

5.4 Discussion

5.4.1 Performance of maize hybrids under field conditions

The study revealed variations in plant height, root mass and grain yield of hybrids between sites. The site x hybrid interaction observed for grain yield is an indicator of the differences in adaptability of the hybrids regardless of nematode infestation levels. The site x hybrid interaction effects recorded for number of root lesions could be explained by the different levels of root fungal infections exacerbated by nematode root damage at the different sites. Sumner *et al.* (1985) reported the interaction of nematodes and *Pythium* spp. fungi to increase root disease severity and decrease yield in maize. Traits such as plant height and root mass were generally higher under nematicide treatment than under nematode infested plots at all sites. These traits are known to improve once nematode populations are very low in most crops. Hybrids which exhibited higher plant heights did not necessarily have significantly lower *P. zeae* populations except for MP709/CML206. Therefore, taller plants are not necessarily nematode free, which justifies the need to count nematodes present in the roots. However, the high *P. zeae* densities recorded in stunted plants confirms reports that nematodes restrain plant growth. These results are consistent with previous observations (Kimenju *et al.*, 1998; Patel *et al.*, 2002; Luc *et al.*, 2005).

Meloidogyne spp. densities were quite low in most of the hybrids compared to *P. zeae* populations. This confirms earlier findings that *P. zeae* is a more aggressive nematode in maize than *Meloidogyne* spp. in Uganda (Talwana *et al.*, 2008; Kagoda *et al.*, 2010a). According to Olowe and Corbett (1976), *P. zeae* has a higher reproductive rate and tolerance to environmentally related stress compared to other nematode species, thus the high densities recorded in the current study.

Hybrids with the highest root mass such as MP709/CML312, CML206/CML444 and CML444/CML206 also had relatively lower *P. zeae* densities (< 6 000 *P. zeae* per 100 g frm) and their yields exceeded 6.0 t ha^{-1}, indicating that these hybrids were resistant to nematodes. Previously, Patel *et al.* (2002) recorded a considerable reduction in root mass and an almost ten-fold increase in *P. zeae* densities in maize inoculated with *P. zeae* indicating that this nematode can cause significant damage especially in susceptible cultivars. Kimenju *et al*. (1998) similarly observed nematodes to cause significant reductions

in root mass of maize OPVs and hybrids. Similarly, *P. zeae* has been reported to limit root growth and eventual yield in rice (*Oryza sativa*) (Prot and Savary, 1993).

Hybrids such as CML206/CML444, MP709/CML312, MP709/CML395, MP709/CML206 and 5057/MP709, which had a relatively lower number of root lesions also registered lower *P. zeae* densities, which confirms the positive correlation between root lesions and *P. zeae* densities. Presence of root lesions is characteristic of damage by root lesion nematodes such as *P. zeae*. These results are consistent with previous observations (Olowe, 1977; Norton and Nyvall, 1999).

More nematodes were recorded at Bufulubi than the rest of the experimental sites. This is probably because sandy soils were more predominant in the experimental site at Bufulubi (61.1%) than the rest (41-49%) of the experimental sites (Chapter 4, Appendix 4.2). Both *P. zeae* and *Meloidogyne* spp. proliferate more in sandy soils than other soil types (Norton, 1978; Dropkin, 1989). The high relative differences in *P. zeae* densities between nematode infested and nematicide treated plots suggest that nematicides are effective in reducing nematode densities. However, they need to be used with precaution due to the risk of environmental pollution and costs of purchasing them. As a result, breeding nematode resistant cultivars is highly advocated. Resistance indices were less than 100 at all sites, which provides substantial evidence that yield losses occurred as a result of nematode damage. However, yield losses due to nematodes manifested more at Namulonge (9.6%) than at Bufulubi (4.8%) probably because of maize being more adapted in Bufulubi than Namulonge (NARO, 2002).

Hybrids such as CML206/CML395 and its reciprocal; MP709/CML206 and CML395/MP709 had lower *P. zeae* populations compared to the resistant check (DK8031). Such hybrids possess genes for resistance *to P. zeae*. *Pratylenchus zeae* resistance is characterized by penetration of fewer *P. zeae*, delayed egg laying and nematode reproduction, less root necrosis and cell wall thickening around the parasitic zone (Kathiresan and Mehta, 2002). The relatively lower *Meloidogyne* spp. observed in hybrids CML206/MP709, CML395/MP709, CML395/CML206 and CML444/CML206 compared to the resistant check can be attributed to inbred lines CML206 and MP709, which exhibited resistance to nematodes (Chapter 4). The inbred line MP709 has also been reported by Williams and Windham (1998) to be *Meloidogyne* spp. resistant. Root-knot resistance is characterized by slow nematode development or no development of juveniles when compared with susceptible hosts (Lawrence and Clark, 1986; Windham and Williams, 1994b).

Grain yield across sites was higher by about 400 kg ha^{-1} under nematicide treated plots than under nematode infestation, a clear indication that nematodes are associated with yield loss in maize. Similarly, yield losses due to nematode damage among hybrids ranged between 1 and 28% compared to a yield loss of 15% in the susceptible cultivar (H614D) across sites. The resistance index was below 100% in hybrids which registered yield losses, which indicates that nematodes played a significant role in reducing grain yield in such hybrids. Hybrids 5057/CML444 and 5057/CML395 had a resistance index of 100% indicating tolerance to nematodes in these hybrids.

Grain yield was high among hybrids which had moderately low nematode densities. Conversely, hybrids whose nematode densities were very low had moderately high grain yields whereas very high yields would have been obtained. This was because inbred lines such as MP709 and 5057 which are nematode resistant had poor adaptation to the local environmental conditions in Uganda hence they contributed to lower yields in their hybrid combinations. The nematode resistant/tolerant CIMMYT lines such as CML444, CML395 and CML206 (as evidenced from the current study, Chapter 4) are also adapted to the tropical conditions in Uganda, and greatly influenced high grain yields. However, the hybrid CML395/MP709 had low nematode populations and had a grain yield of 7.0 t ha^{-1} under nematode infestation. It therefore combined nematode resistance and adaptation to the environment.

The negative correlations and regression coefficients recorded between grain yield and number of root lesions, grain yield and *P. zeae* densities, and grain yield and *Meloidogyne* spp. densities are evidence that nematodes are associated with reduced grain yield in susceptible maize cultivars. Similarly, Tarte (1971) found a highly significant negative correlation between numbers of *P. zeae* and yield of maize. However, even with low nematode densities (under nematicide treatment), negative correlations were observed between grain yield and the nematode densities. It is therefore important to use management practices which completely give the maize plant an advantage over the nematodes, such as breeding for resistant cultivars. However, the coefficient of determination (R^2) as computed from the regression analysis was very low (0.1-22%) signifying that some variation in the model could not be explained by the nematode treatments alone but other factors too.

5.4.2 Response of the F_1 hybrids to *P. zeae* infection in the screenhouse

Though data from screenhouse experiments almost corresponded with that from the field experiments, plant height and root mass were lower in the screenhouse experiment. This

was expected since screenhouse experiments have limitations in space and time. Hybrids MP709/CML312 and CML395/CML312 maintained the highest plant heights in the screenhouse and in the field. These hybrids were, therefore, least affected by the presence of nematodes. Zsuzsanna *et al.* (2002) described plant height as a genetically complex but environmentally stable trait. Taller hybrids produce a higher dry matter yield but the translocation rate of assimilates to the kernels of taller hybrids is lower than for shorter hybrids (Begna *et al.*, 2000).

Although there was a difference in root mass in the uninoculated pots compared to the *P. zeae* inoculated pots, it was by a small margin. This can be attributed to the short duration of the experiment, but also the limited expansion space in the pots. However, Oyekanmi *et al.* (2007) similarly reported a higher root mass in *P. zeae* inoculated pots than the controls. According to Evans (1982) and Haverkort *et al.* (1994), roots may proliferate at a higher rate to absorb nitrogen in the subsoil depleted by nematodes in the topsoils leading to high root mass in nematode infested plots than the non-infested. Similarly, roots can be stimulated to produce at sub-threshold levels, increasing root production despite nematode infection ([4]Coyne, D., personal communication), which is a characteristic of nematode tolerant cultivars.

Mean *P. zeae* density was 44 007 per 100 g frm in the inoculated pots, which indicates that the nematodes increased by 8.8-fold in the two months the experiment was conducted. However, the five most resistant hybrids had *P. zeae* densities far below the mean (< 6 000 *P. zeae* per 100 g frm) and RF of less than 1.5. This demonstrates that a nematode resistant hybrid should have the capacity to reduce entry and rapid multiplication of nematodes in its root system. According to Kathiresan and Mehta (2002), nematode penetration in resistant crops is reduced by mechanical (lignin like substances) and biochemical barriers present in the plant. A number of *P. zeae* resistant hybrids in the screenhouse trial recorded high grain yields in the field when compared to the resistant check. These included: 5057/MP709, 5057/CML444, CML395/CML312, MP709/CML312 and CML312/CML206. This indicates that screenhouse data was quite reliable in explaining performance in the field. Similar observations were reported by Speijer and De Waele (1997).

5.4.3 Heterosis and relative yield

Heterosis was computed for *P. zeae* under screenhouse and field conditions, and for *Meloidogyne* spp. and yield under field conditions. Hybrids CML206/CML312,

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CML395/CML312, CML312/5057 and MP709/CML312 had resistance to *P. zeae* as evidenced by the negative heterosis in the screenhouse and in the field. For *Meloidogyne* spp., negative heterosis was recorded for the hybrids CML395/MP709, CML395/CML206 and CML312/CML444 in the field. Inbred lines MP709, 5057, CML206, and CML444 evidently have genes for *P. zeae* resistance and tolerance (CML444) (Chapter 4), which explains the negative heterosis observed in their hybrid combinations. Likewise, field and screenhouse evaluation provided evidence that inbred lines MP709, 5057 and CML444 have genes for resistance to *Meloidogyne* spp. Remarkably, these are dominant or epistatic genes for *P. zeae* and *Meloidogyne* spp. resistance since dominance and epistasis are the underlying genetic basis for heterosis (Falconer, 1981; Chapter 4). According to Cromley *et al*. (2002), single cross hybrids would have an adequate level of resistance if at least one parent has resistance. Likewise, the best hybrid vigour is obtainable when crosses are made between parents originating from genetically different populations (Hallauer and Miranda, 1988), which seems to be the case in this diallel population. It is hence the differences in allele frequencies between parents which are responsible for the expression of heterosis (Ricardo and Filho, 2003).

Hybrids MP709/CML312, 5057/CML206, CML312/5057, CML206/CML395, CML312/CML206, CML395/CML312 and CML312/CML395 had the highest heterosis for grain yield and *P. zeae* resistance under nematode infestation, based on averages from field and screenhouse data for *P. zeae*. This is an indication that these hybrids were superior in yield and *P. zeae* resistance to the mid-parent, susceptible parent or resistant parent. Hybrids CML206/MP709, CML395/MP709, CML395/CML206 and CML312/CML444 exhibited the highest heterosis for grain yield and *Meloidogyne* spp. resistance under field conditions. Hybrid CML395/5057 and its reciprocal had high heterosis for grain yield despite poor heterosis for *P. zeae* and *Meloidogyne* spp. resistance. This means that this hybrid is tolerant to nematode infection. According to Falconer (1981), the heterosis observed among hybrids and their reciprocals is a result of the contribution from the maternal and non-maternal components. Unfortunately, heterosis that confers high yield in F_1 hybrids declines sharply by over 50% in the F_2 and subsequent generations (Falconer, 1981). Farmers can hence plant F_1 seed if they are to maximise yields, but not from the subsequent generations. Significant reciprocal differences in grain yield observed for hybrids MP709/5057 and CML444/CML312 under nematode infestation could be attributed to maternal and non-maternal effects, respectively, as reported in Chapter 4. The significant maternal effects consistently resulted from inbred MP709. The wider range in heterosis for grain yield among hybrids under nematode infestation than under nematicide treatment suggests that hybrid vigour declines sharply among nematode susceptible maize hybrids compared to nematode

resistant hybrids. The change in rank order for grain yield observed in most of the hybrids, based on Spearman's rank correlation, under nematode infestation when compared to nematicide treatment plots further confirms that hybrid performance can be affected by the presence of nematodes.

Under nematode infestation, only 16 hybrids had higher relative yield compared to the mean of both checks, the best check and the trial mean, whereas it was 20 hybrids under nematicide treated plots. This implies that some hybrids were unable to express maximum relative yield under nematode pressure since they lack genes for resistance to nematodes. Therefore, hybrids such as CML395/MP709, CML312/5057, CML312/CML206, CML312/CML444, CML395/CML312 and CML312/CML395 would be recommended for advancement in breeding programmes since they were high yielding, had high mid-parent heterosis, and high relative yield compared to the checks and the trial mean under nematode pressure. These hybrids would be advanced by testing them for adaptability across more environments and eventual release.

5.5 Conclusions

The following conclusions were drawn from the study:

- Based on the reproductive factor, 24 hybrids were *P. zeae* susceptible whereas six hybrids were resistant to *P. zeae* under screenhouse conditions.
- Grain yield across locations was higher by about 400 kg ha^{-1} under nematicide treated plots than under nematode infestation. The nematode resistant hybrids exhibited high yields ranging from 5.0 to 8.4 t ha^{-1} compared to 5.0 t ha^{-1} exhibited by the best check.
- Grain yield loss ranged between 1 and 28% among susceptible hybrids, indicating high levels of damage by nematodes.
- Under field conditions, favourable heterosis was recorded on 18 hybrids for *P. zeae*, and on three hybrids for *Meloidogyne* spp.
- Under nematode infestation, only 16 hybrids had higher relative yield compared to the mean of both checks, the best check and the trial mean, whereas it was 20 hybrids under nematicide treated plots.

The hybrids which were high yielding, had high mid-parent heterosis, and high relative yield compared to the checks and the trial mean under nematode pressure were: CML395/MP709, CML312/5057, CML312/CML206, CML312/CML444, CML395/CML312 and CML312/CML395. These hybrids and their parents will be used in breeding programmes in Uganda to develop new nematode resistant cultivars.

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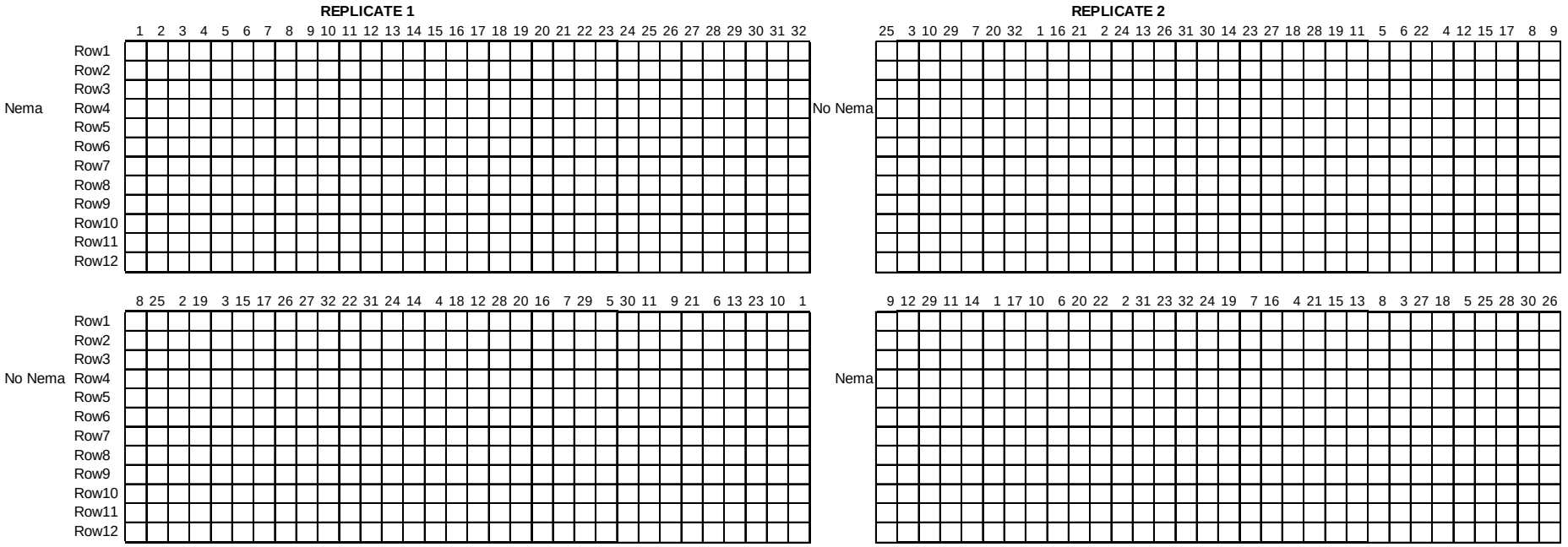
Appendices

Appendix 5.1: Screenhouse split plot layout for evaluation of F_1 hybrids for resistance to *P. zeae*



Each box, i.e.☐, represents a plastic pot (with 2 500 litres of soil) where the maize seeds were planted. No nema = Pots where soil was sterilised and nematicide was applied. Nema = Pots inoculated with 5 000 P. zeae. Hybrids evaluated include: 1) MP709/5057, 2) MP709/CML206, 3) MP709/CML444, 4) MP709/CML395, 5) MP709/CML312, 6) 5057/MP709, 7) 5057/CML206, 8) 5057/CML444, 9) 5057/CML395, 10) 5057/CML312, 11) CML206/MP709, 12) CML206/5057, 13) CML206/CML444, 14) CML206/CML395, 15) CML206/CML312, 16) CML444/MP709, 17) CML444/5057, 18) CML444/CML206, 19) CML444/CML395, 20) CML444/CML312, 21) CML395/MP709, 22) CML395/5057, 23) CML395/CML206, 24) CML395/CML444, 25) CML395/CML312, 26) CML312/MP709, 27) CML312/5057, 28) CML312/CML206, 29) CML312/CML444, 30) CML312/CML395, 31) DK8031, and 32) H614D.

CHAPTER SIX

Response to two cycles of S_1 progeny recurrent selection for nematode resistance in three tropical maize populations

Abstract

Plant-parasitic nematodes are a key constraint to maize production leading to economic grain yield loss in susceptible cultivars. Open pollinated maize varieties (OPVs) carry favourable genes for nematode resistance but this resistance has never been exploited in breeding programmes. Two cycles of S_1 progeny recurrent selection were therefore performed separately for three tropical OPVs in nematode infested fields with the aim of improving nematode resistance and subsequently grain yield. The original (cycle 0) and advanced populations (cycle 1-susceptible, cycle 1-resistant and cycle 2-resistant) were evaluated at three sites using a split plot design with three replications. The design had the main plot factor as the nematode treatments (nematode infested/nematicide treatment) and the sub-plot factor as the maize population cycles. The S_1 progeny recurrent selection was highly effective in improving nematode resistance and subsequently grain yield in the three maize populations. Each cycle of selection for nematode resistance improved grain yield by 200 to 600 kg ha^{-1} in the three maize populations. Actual net gains in grain yield after two cycles of selection relative to cycle 0, were 6.3%, 10% and 22% for the populations Longe 1, ZM521 and Longe 4, respectively. The *P. zeae* densities reduced by 57%, 59% and 55% in Longe 1, Longe 4 and ZM521, respectively, after the two cycles of selection. For *Meloidogyne* spp., net reductions were 65%, 39% and 59% for Longe 1, Longe 4 and ZM521, respectively. Net reductions in the number of root lesions were 16%, 28% and 40% for Longe 1, Longe 4 and ZM521, respectively. Realized heritability (h^2) for *P. zeae* and *Meloidogyne* spp. ranged from 66-96% at cycle 2. For grain yield, h^2 ranged from 50-83% at cycle 1 and 80-86% at cycle 2. Broad sense heritability (H^2) for grain yield at cycle 2 ranged from 74-97% for the three maize populations. Therefore, the two cycles of S_1 progeny recurrent selection improved grain yield in the three maize populations through reduction of nematode densities. A possibility exists for further improvement in nematode resistance with additional cycles of S_1 recurrent selection. The current study is a baseline for future studies aimed at improving nematode resistance in maize with a recurrent selection approach.

Keywords: Grain yield, heritability, *Meloidogyne* spp., Open pollinated maize varieties, *Pratylenchus zeae*, S_1 progeny recurrent selection**.**

6.1 Introduction

Maize is the most important cereal crop in Uganda providing over 40% of the population's calories with an annual consumption of about 23 kg per capita per year (NARO, 2002). Maize also serves as a cash crop to both rural and urban communities, with informal exports equivalent to 14-20% of total production (Magnay, 2004). However, maize yields are low in Uganda, fluctuating between 0.8 and 1.5 t ha^{-1} (Kasenge *et al.*, 2001; FAOSTAT, 2009). Majority of the biotic and abiotic constraints responsible for the fluctuating yields are continually being addressed and strategies put in place to ensure high grain yield of maize. However, little has been done to address the impact of plant-parasitic nematodes on maize in Uganda although they are abundant, and have been associated with yield loss (Butseya *et al.*, 2005; Kagoda *et al.*, 2010a).

Pratylenchus zeae Graham and *Meloidogyne* spp. Goeldi are the most damaging nematodes of maize in Uganda (Butseya *et al.*, 2005; Talwana *et al.*, 2008; Kagoda *et al.*, 2010a). *Pratylenchus zeae* particularly causes a mechanical breakdown of cells and necrosis of stellar and cortical tissues, resulting in formation of cavities (Olowe and Corbett, 1976; Olowe, 1977). *Meloidogyne* spp. penetrate roots of plants near the growing points, feeding on the giant cells which thicken and become saccate causing galls in infected roots (Williams, 1972). However, typical gall symptoms may be absent on maize despite infection (Idowu, 1981; Riekert, 1995; Asmus *et al.*, 2000). To overcome the nematode production constraint on maize, exploitation of host plant resistance (HPR) is highly advocated. This is because it is cheap, safe, and poses no technical difficulties to the farmer, provided that resistance genes are readily available (Trudgill, 1991). A highly nematode resistant crop has also been found to provide at least two years of nematode control benefit when used in rotation (Roberts, 2002). However, resistant cultivars are currently not available in Uganda, and are also scarce in other places.

The recurrent selection breeding scheme offers an effective means of increasing the frequency of favourable alleles of economic value in genetically broad based cross-pollinated populations (Sprague and Dudley, 1988). These populations can then serve as a potential source of superior inbreds and can inhibit development of a possible genetic ceiling for future hybrid improvement (Duvick, 1992; Kannenberg and Falk, 1995). Intrapopulation recurrent selection is effective for improving pest resistance, adapting germplasm for specific environments, and changing the genetic composition of the grain (Hallauer and Miranda, 1988). In the absence of overdominance, S_1 or S_2 progeny recurrent selection is considered to be superior to other methods of recurrent selection for improvement of the population per se (Lamkey, 1992). Alleles are fixed rapidly and deleterious, homozygous alleles are

exposed and eliminated early in selection (Weyhrich *et al*., 1998). The S_1 progeny recurrent selection is characterised by additive genetic effects with partial to complete dominance, which are of greater importance than non-additive effects in maize populations (Hallauer *et al.*, 1988). Replicated S_1 progeny evaluation trials for selecting resistant genotypes are also highly associated with higher heritabilities compared to S_0 plants (Hallauer and Miranda, 1988). S_1 progeny selection is effective in increasing the level of pest resistance, vigour, standability and yield through the generated hybrid seed from single crosses or through the improved OPVs. It has also been used to modify ear and plant heights as well as reduce ear rot and root lodging in maize (Ramírez-Díaz *et al.*, 2000). Zaidi *et al.* (2004) reported decreased ear abortion and increased assimilate supply during grain filling in a study to improve maize populations to drought tolerance using S_1 families. Edmeades *et al.* (1999) reported 38% increase in grain yield after three cycles of S_1 recurrent selection in water stressed environments.

Some work has been done on nematode resistance using recurrent selection breeding techniques but not necessarily on maize, and mostly on sedentary nematodes. For example, through recurrent selection and progeny testing, five bitter almond genotypes indicated dominance of resistance to *Meloidogyne javanica* (Kochba and Spiegel-Roy, 1975). After seven cycles of recurrent selection, partial resistance was achieved against *M. trifoliophila* in white clover (Mercer *et al.*, 2000). In S_1 evaluations of pearl millet, each of the four cultivars was heterogeneous for resistance to *M. incognita* but the progeny varied from highly resistant to highly susceptible (Timper and Wilson, 2006). The S_1 recurrent selection improves a trait by exploiting the additive genetic effects, therefore a suitable approach for maize population improvement. However, selection for nematode resistance has not been conducted in maize yet nematodes reduce yields in maize fields, especially on sandy soils, which are prevalent throughout Sub-Saharan Africa.

The objectives of the study were to:

(i) improve nematode resistance in three maize populations using S_1 progeny recurrent selection; and

(ii) determine the net reduction in nematode populations and resulting gain in grain yield in the three maize populations following selection for nematode resistance,

The hypothesis tested was that nematode resistance could be improved through two cycles of S_1 progeny recurrent selection and a significant increase in grain yield could be realised.

6.2 Materials and methods

6.2.1 Study area and germplasm description

Initial selection, evaluation and recombination trials were conducted at the International Institute of Tropical Agriculture (IITA), Namulonge in Uganda. Evaluation of all the cycles was conducted at three sites, namely; Namulonge (1 200 m above sea level (a.s.l); 0°32'N, 32°34'E; 1 300 mm bimodal rainfall), Kabanyolo (1 150 m a.s.l; 0° 28'N, 32° 37'E; 1300 mm bimodal rainfall) and Bufulubi (1 130 m a.s.l; 00° 49' N, 033° 42' E; 1 345 mm bimodal rainfall), which are characterized by greater than 40% sandy soils (Wortmann and Eledu, unpublished data; Chapter 4, Appendix 4.2).

Three OPVs namely Longe 1, Longe 4 and ZM521 (Table 6.1) were used in the S_1 progeny recurrent selection. Longe 1 is an OPV released in 1991 by the National Agricultural Research Organisation (NARO) in Uganda. It matures in 120 days, yields up to 5 t ha^{-1} and is resistant to maize streak virus (MSV) (NARO, 2002). Longe 4 is an OPV released by NARO in Uganda. It matures in 105 days with a potential yield of 4 t ha^{-1}, and is resistant to drought and MSV. Farmers in eastern and southern Uganda reported low yields (1-2 t ha^{-1}) of Longe 1 and Longe 4 compared to a potential of 4-5 t ha^{-1} (Kagoda *et al.,* 2010a). Similarly fewer farmers are involved in cultivation of these OPVs compared to other OPVs like Longe 5. It was for these reasons that these cultivars were included in the study. ZM521 is a mid-altitude medium maturing line resistant to MSV and stem borers, which was developed at the International Maize and Wheat Improvement Centre (CIMMYT) in Zimbabwe. It is a synthetic of B and A lines from the CIMMYT programme and it is widely grown throughout East and Southern Africa. For this reason, it was included in the study.

Table 6.1: Profiles of the germplasm used in the recurrent selection process

Maize population	Origin	Kernel colour	Variety type
Longe 1	Uganda	White	Improved
Longe 4 (LP16)	Uganda	White	Improved
ZM521 (VP05153)	CIMMYT-Zimbabwe	White	Improved

6.2.2 Formation of S_1 lines

The recurrent selection procedure was applied separately to each of the three OPVs, which consitituted the source populations cycle 0 (C_0) Longe 1, C_0 Longe 4 and C_0 ZM521. Selection nurseries were established at IITA-Namulonge farm between November 2007 and March 2008 in fields previously densely planted with maize since they contained a reservoir

of nematodes (> 500 *P. zeae* and > 100 *Meloidogyne* spp. per 100 g of soil) and their eggs from the previous seasons. Each of the C_0 populations (500 plants per population) was sown in rows in field isolation blocks (Table 6.2; Fig. 6.1). One seed was sown per hill, and recommended agronomic practices were followed. At flowering 300 to 400 promising plants in each population were self-pollinated to generate S_1 ears. Plants for selfing were selected based on visual observations of the traits below:

i) Pest and disease resistance. The key pests selected against were the stem borers (mainly *Busseola fusca* and *Chilo partellus*), termites (mainly *Macrotermes* spp. and *Amitermes* spp.) and nematodes based on observed symptoms. Nematode symptoms selected against were: stuntedness, chlorosis and general paleness of the plant, reduced plant vigour, and low grain yield at harvest. Diseases selected against were turcicum leaf blight (TLB), caused by *Exserohilum turcicum*; grey leaf spot (GLS), caused by *Cercospora zea-maydis*; MSV, caused by a geminivirus transmitted by leaf hoppers of the genus *Cicadulina*; ear rots, caused by *Fusarium* and *Diplodia* spp.; rusts caused by *Puccinia sorghi* and *P. polysora*; phaeosphaeria leaf spot (PLS), caused by *Phaeosphaeria maydis*; and head smuts caused by *Sphacelotheca reliana.*
ii) Ear size – medium to large.
iii) Earliness – early to medium maturing (90 to 110 days to physiological maturity).
iv) Ear height – Short (80 cm to 100 cm).

After harvesting and threshing the S_1 plants, 42 S_1 families with healthy white flint kernels, high grain mass and sufficient seed were selected in preparation for field evaluation for each of the source populations. The rank summation selection index method (Mulamba and Mock, 1978) was used as the criteria for selection. This involved ranking each line (1 to n) for each of the traits measured (grain mass, kernel size and kernel texture) and calculating the index by summing the trait ranks for each line. It should be noted that nematode resistance vigour among plants selected at this stage was based only on phenotypic observations such as the general vigour of the plant or seed.

6.2.3 *Pratylenchus zeae* and *Meloidogyne* spp. inoculum preparation

Pratylenchus zeae used for inoculation were initially extracted using a modified Baermann sieve method (Coyne *et al*., 2007) from infected maize roots, obtained from farmers' fields in Iganga District, Uganda. The *P. zeae* were multiplied on carrots (*Daucus carota* L.), cv. Nantes in the laboratory (Kagoda *et al.*, 2010b), and used for inoculating the screenhouse

trials. The *P. zeae* culture was also maintained on the susceptible maize hybrid H614D in pots in a shadehouse at IITA-Namulonge farm. For *Meloidogyne* spp., galled tomato roots were used for egg production (Hussey and Barker, 1973) and generation of juveniles for inoculation. These were also maintained in pots in the shadehouse and used to inoculate the trials. Where nematode populations were found to be low in the field (e.g., in Namulonge during the final evaluation trial), maize root pieces infested with mainly *P. zeae* and *Meloidogyne* spp. were used as inoculum.

6.2.4 Field evaluation of S_1 families and generation of nematode resistant C_1 seed

Field evaluation of the 42 S_1 families for nematode resistance and susceptibility, and for each of the three source populations was carried out at IITA-Namulonge farm between April and August 2008 (Table 6.2). Two previously selfed checks were included forming 44 S_1 families for evaluation. The checks were OPVs of Gandajika 8022 and Western Yellow, which are susceptible and resistant to *Pratylenchus zeae*, respectively (Oyekanmi *et al.*, 2007). The 42 S_1 families from each source population were separately evaluated in a split plot design with two replications (Appendix 6.1). Nematode treatment was the whole plot factor with two levels (Nematode infested and nematicide treated plots), while the S_1 progeny of the 42 selected C_0 plants plus two checks were the 44 levels of the subplot factor laid out randomly in single rows, with 15 plants per row, in an 11 x 4 α-lattice design. Plant spacing was maintained at 75 x 30 cm. The nematode infested plots comprised an average Pi of 500 *P. zeae* and 100 *Meloidogyne* spp. per 100 g of soil per plot and lesser populations of other nematode species. Fenamiphos (nemacur™), a non-volatile nematicide was applied at a rate of 2.5 kg ha^{-1} (≈ 2.3 g per plant) and incorporated 5-8 cm deep with a hand hoe prior to planting in the nematode protected plots. Planting was ear–to-row to maintain genetic purity of the S_1 families. Out of the 15 plants in each row, five were randomly selected and used for root nematode assessment and the rest (10 plants) were used for grain yield assessment. Data collected included *P. zeae* densities, *Meloidogyne* spp. densities, grain yield, anthesis-silking interval, root mass and number of root lesions. Selection of lines from each replicated trial for recombination was based on a weighted

selection index that involved initial standardization of the values for the selected traits using the formula: $\frac{\text{Value of individual line - Mean of all the lines}}{\text{Standard deviation of all the lines}}$

Index scores were calculated as I = (-10Pz - 8Melo + 5YldNe + 4YldNo – 3ASI + 3Rtms – 3Lsns), where Pz = *P. zeae* densities in nematode infested plots, Melo = *Meloidogyne* spp. densities in nematode infested plots, YldNe is grain yield in nematode infested plots, YldNo is grain yield in nematicide treated plots, ASI is anthesis-silking interval in nematode infested plots, Rtms is root mass in nematode infested plot, Lsns is number of root lesions in nematode infested plots. Therefore, following evaluation of the 42 S_1 progeny families from each source population, remnant selfed seed (i.e., S_1 lines of 20 C0 plants from the selected 42 Co plants per source population) with S_1 progeny exhibiting resistance to nematodes and other traits in the selection index were planted and subsequently randomly mated (Fig. 6.1). In addition, remnant selfed seed (S_1 lines) of 10 C_0 plants (from the remaining 22 of 42 C_0 plants) with S_1 progeny exhibiting susceptibility to nematodes was also planted (divergent selection) and subsequently randomly mated. The 20 S_1 lines with resistance to nematodes represented 47.6% (approximately 0.838 selection intensity) of the 42 C_0 plants originally selected from each source population. The 10 S_1 lines with susceptibility to nematodes represented 23.8% (approximately 1.295 selection intensity) of the 42 C_0 plants originally selected per source population. The 20 nematode resistant and 10 nematode susceptible S_1 lines per source population were sown ear-to-row in separate isolation blocks for random mating between November 2008 and February 2009 (Table 6.2; Fig. 6.1). The 20 nematode resistant S_1 lines were sown in 20 rows of 30 hills, giving a plant population of 600 plants in each population. In a separate isolation block, the 10 nematode susceptible S_1 lines were sown in 10 rows of 30 hills giving a plant population of 300 plants in each maize population. Plant spacing used in the recombination nurseries was 80 x 40 cm.

The 20 S_1 lines in the nematode resistant population and the 10 S_1 lines in the nematode susceptible population were randomly mated by hand using bulked pollen from the S_1 plants to pollinate silks of all S_1 lines within each respective population. At maturity, the seed from

the ears within each of the 20 S_1 nematode resistant lines were harvested, shelled, dried and bulked so as to maintain the pedigree of each S_1 nematode resistant line and the resultant 20 C_1R progeny lines. Similarly, seed from the ears within each of the 10 S_1 nematode susceptible lines were harvested, shelled, dried and bulked. Equal sub-samples of seed from each of the 20 S_1 nematode resistant lines and the 10 S_1 nematode susceptible lines were composited to form cycle 1-resistant (C_1R) seed and cycle 1-susceptible (C_1S) seed, respectively. This procedure was repeated with the best 10 nematode resistant lines to generate cycle 2-resistant (C_2R) seed but ensuring that seed within each of the 10 S_1 nematode resistant lines is separately reserved. The C_1S seed were stopped at that population level for latter use as a check.

The method of rank summation selection index (Mulamba and Mock, 1978) was used in the selection process to facilitate the identification of superior families for recombination for the next cycle, as well as, to maximise gain from selection.

6.2.5 Field and screenhouse evaluation of C_1 seed and recombination of nematode resistant C_2 seed

Field and screenhouse evaluation of the 20 C_1 lines for resistance to *P. zeae* and *Meloidogyne* spp. was conducted at IITA-Namulonge farm between March 2009 and June 2009 (Table 6.2; Fig. 6.1). Four cultivars used as checks were the C_0 line (for each population), Gandajika 8022 (susceptible to *P. zeae*), Western Yellow (both *P. zeae* and *Meloidogyne* resistant) and *Meloidogyne* spp. susceptible lines from the S_1 selection (named S_1-5, S_1-201 and S_1-91 for Longe 1, ZM521 and Longe 4, respectively). The field and screenhouse experimental designs were split plot with two replicates. In the field, nematode treatment (nematode infested vs nematicide treatment) was the whole plot factor while the C_1 lines were the subplot factor arranged in a 6 x 4 α-lattice design (Appendex 6.2). Double rows were planted per genotype, each composed of 16 plants spaced at 75 cm between rows and 30 cm between plants in a row. A total of 32 plants were thus maintained in each genotype in the field, of which 12 were used for root nematode assessment and the rest (20 plants) used for yield assessment. The screenhouse type used for evaluation was a detached Quonset greenhouse with curved metallic rafters, and roofing made of twin polycarbonate material. The sides were fitted with transparent, rain-proof netting. The average temperature and relative humidity estimates in the screenhouse were 25.5^{o}C and 65%, respectively. Sterilized loam and sand soils were used in the screenhouse. These were mixed in equal proportions to form about 2 500 ml of soil sufficient to fill the plastic pots. For each line in the screenhouse trial, 12 pots were prepared and each pot sown with two seeds, later thinned to one seedling one week after planting (Appendix 6.3). Separate trials were

conducted for *Meloidogyne* spp. and *Pratylenchus* spp. evaluation in the screenhouse. Each pot was inoculated with 5 000 adults and juveniles of *Meloidogyne* spp. and *Pratylenchus zeae*. The inocula were administered using 10 ml pipette to ensure uniformity of application. Data collected from the field and screenhouse trials was the basis upon which the best 10 nematode resistant C_1R lines were selected.

Remnant C_1R seed from 10 of the 20 S_1 nematode resistant lines with superior C_1R progeny tests (field and screenhouse trials) were planted (23.8% of the 42 C_0 plants originally selected; approximately 1.295 selection intensity). Planting was done in single rows of 30 hills per line to form a total of 300 plants per population spaced at 80 x 40 cm. Recombination of these 10 lines was done at flowering per population by bulking the pollen and randomly mating the plants in all possible combinations between November 2009 and February 2010. Cycle 2-nematode resistant (C_2R) seed was generated from this recombination. According to Weyhrich *et al.* (1998), genetic drift becomes a stronger force in altering allele frequencies than selection when fewer than 10 lines are recombined.

6.2.6 Multi-locational field evaluation of the original and advanced S_1 progeny

Evaluation of C_0, C_1R, C_1S and C_2R populations for nematode resistance was conducted in three-replicate trials at Namulonge (1 200 m above sea level (a.s.l); 0°32'N, 32°34'E; 1 300 mm bimodal rainfall), Kabanyolo (1 150 m a.s.l; 0° 28'N, 32° 37'E; 1300 mm bimodal rainfall) and Bufulubi (1 130 m a.s.l; 00° 49' N, 033° 42' E; 1 345 mm bimodal rainfall) between February 2010 to July 2010 (Table 6.2; Fig. 6.1). The design was a split-plot at all sites with whole plots being the levels of nematode treatment (nematode infested vs nematicide treated plots); and the subplot levels were the original and advanced seed cycles (C_0, C_1R, C_1S and C_2R) (Appendix 6.4). The nematode infested plots comprised an average Pi of 500 *P. zeae* and 100 *Meloidogyne* spp. per 100 g of soil per plot and lesser populations of other nematode species. Each subplot (4.2 x 2.8 m^2) was made up of five rows of 15 hills, providing a plant population of 75 plants per sub-plot spaced at 0.75 m between rows and 0.3 m within rows. Of the 75 plants in each sub-plot, 15 were used for nematode assessment through destructive sampling, whereas the remaining 60 plants were used for assessment of agronomical traits and grain yield. Screenhouse evaluation was not conducted for the multilocation trial to compare performance of original and advanced populations.

Table 6.2: Time line for the recurrent selection activities

Season	Activity
November 2007 – March 2008	500 plants per original (source) population of Longe 1, Longe 4 and ZM521 planted at IITA-Namulonge for selfing.
April 2008 – August 2008	Field evaluation for nematode resistance and susceptibility of the selected 42 S_1 progeny per population. Selection of 20 nematode resistant and 10 nematode susceptible progeny for advancement using their remnant seed.
November 2008 – February 2009	Remnant seed of both resistant and susceptible S_1 lines sown ear-to-row. Random mating of S_1 lines in nematode resistant and susceptible populations by bulking pollen and making crosses in all possible combinations. Some of the harvested seed from each of the 20 lines was kept separately for later use in initiating C2 seed; the remaining seed was bulked to form C_1R and C_1S seed.
March 2009 – June 2009	Remnant seed of the individual 20 lines comprising the nematode resistant C_1R families subjected to field and screenhouse evaluation for *P. zeae* and *Meloidogyne* spp. resistance at IITA-Namulonge for each of the 3 populations. 10 progeny with the highest weighted selection index picked for advancement to C_2R using their remnant seed.
November 2009 – February 2010	Planting of remnant seed of the 10 selected progeny, pollen bulked and plants randomly mated but reserving some seed from each of the 10 lines. C_2R seed generated from this recombination.
February 2010 – July 2010	Field evaluation of C_0, C_1R, C_1S and C_2R done in 3 replicate trials at Kabanyolo, Bufulubi and Namulonge for each of the three maize populations.

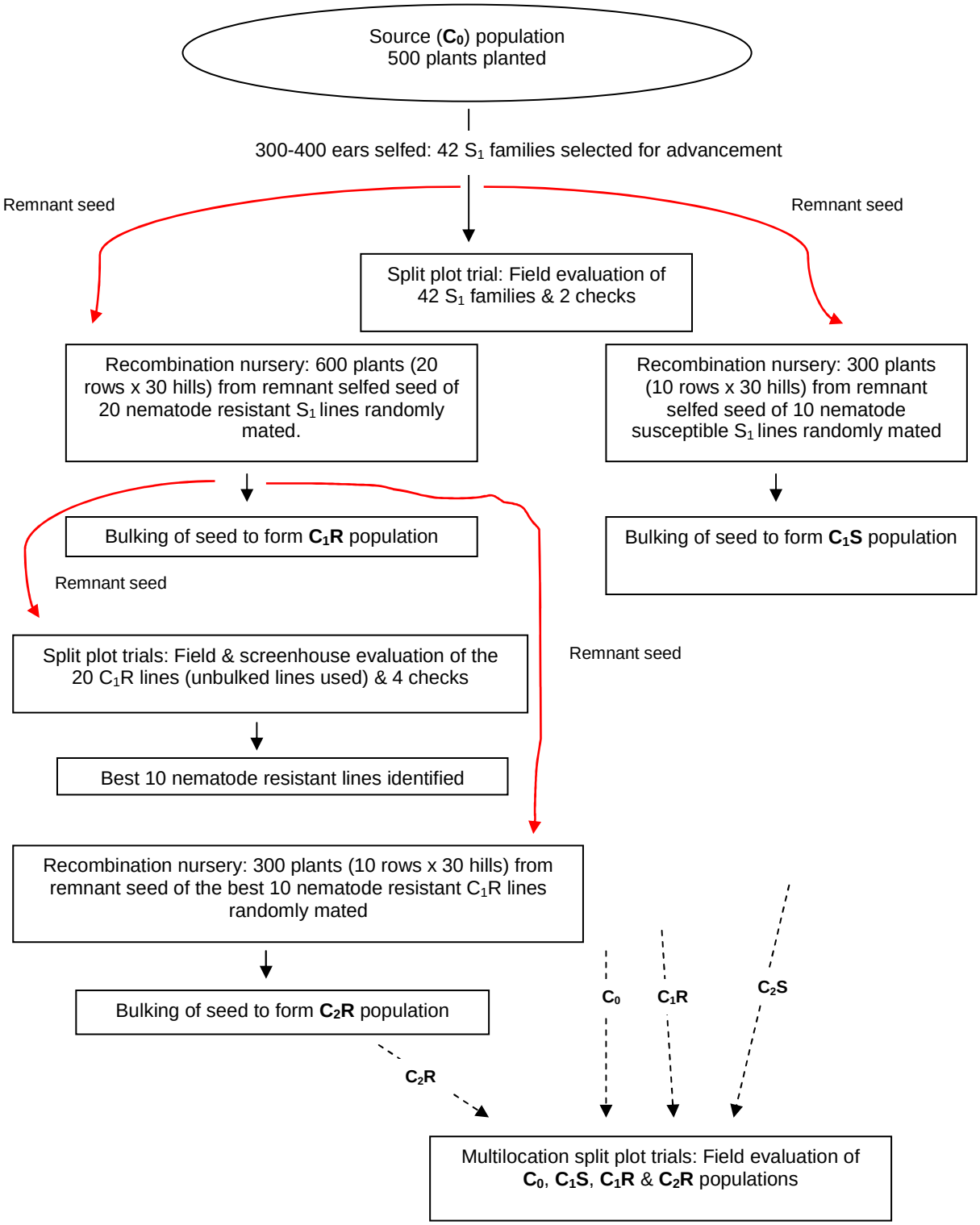


Figure 6.1: Flow diagram for the selection scheme applied to a single source population

6.2.7 Nematode damage and population density assessment

For all the field experiments in the three sites, soil samples from each plot were collected for nematode (vermiform) population counting by species at or shortly before planting (P_i). The soil samples were collected in each plot using a trowel at a depth of 15 cm, discarding the

upper 5 cm (Todd and Oakley, 1996; Coyne *et al.*, 2006). About 10 soil sub-samples per plot were combined to form one sample. From 50% flowering, root samples were taken from the root system of 15 randomly selected plants in each plot for final nematode (P_f) assessment in the field. In the screenhouse, all plants were carefully uprooted at the flowering stage for nematode assessment. At this stage, the nematodes were expected to have completed two generations (Taylor and Sasser, 1978; Windham and Williams, 1987; Dropkin, 1989).

The soil samples and, later on, the root samples obtained from the field were brought to the nematology laboratory. The roots were washed clean with tap water and their mass taken. Root lesions were then counted visually per 5 g of fresh roots. The soil samples were cleaned of all debris, stones, and the big soil lumps were broken to fine particles. The soil was then poured on to a plastic plate for thorough mixing. Nematodes were extracted from a 100 ml soil sub-sample (P_i), and from a macerated 5 g fresh root mass (frm) sub-sample (P_f), using a modified Baermann sieve method (Coyne *et al.,* 2007). The samples were examined after a 48 hour extraction period, and nematodes counted using a stereomicroscope. Both P_i and P_f were estimated from three x 2 ml aliquots (taken from a 25 ml suspension). Nematode densities in the roots at flowering (P_f) were standardized for every sub-plot by subtracting the nematode densities from the soil obtained at time of planting (P_i), i.e., $P_f - P_i$, as reported by Forrest and Holliday (1979). In the screenhouse, Oostenbrink's (1966) reproduction factor, $RF = P_f/P_i$, was used to assess resistance to nematodes, with $RF \leq 1.5$ indicating resistance to nematodes, $1.5<RF\leq2.0$ indicating moderately resistant, $10\geq RF>2.0$ indicating susceptibility and $RF>10$ indicating very susceptible to nematodes (Ferris *et al.,* 1993). The RF was not estimated for the field nematodes because of the variations in P_i in the field plots, as a result of many biotic factors.

6.2.8 Assessment of agronomic traits and grain yield

In the field, plant height and ear height measurements were recorded after flowering. Plant height was measured as height from the base of the plant to the insertion of the first tassel branch of the same plant, whereas ear height was measured as height between the base of a plant to the insertion of the top ear of the same plant (Magorokosho *et al.*, 2007). The number of days to mid-silking and anthesis were estimated as number of days from planting to 50% plants with silk emerged and tassels shedding pollen, respectively. Before harvest, the number of plants per plot was recorded to enable prolificacy assessment and calculation of grain yield. Grain yield was taken on an entire plot basis at harvest and later adjusted to 12.5% moisture (CIMMYT, 1985) using the formula:

$$\text{Grain Yield (t ha}^{-1}\text{)} = \frac{\text{Grain weight (kg/plot) x 10 x (100 - Grain moisture content)}}{87.5 \diagup \text{Plot area}}$$

In the screenhouse, root damage was assessed from fresh root mass, number of root lesions and galls per 5 g of fresh root mass. However, root lesions were assessed only in *P. zeae* inoculated trials whereas root galls were assessed only in *Meloidogyne* spp. inoculated trials. This is because *P. zeae* infection causes root lesions or necrotic roots but does not cause root galls. Conversely, *Meloidogyne* spp. causes root galls but does not cause root necrosis.

6.2.9 Statistical analyses

Test for normality of the data was performed using the Proc Univariate Normal Plot procedure in SAS version 9.1.3 (SAS, 2004). Transformations were performed to achieve normality using log (x+10) for root mass, number of root lesions, anthesis to silking interval (ASI), and *P. zeae* densities. *Meloidogyne* spp. densities were transformed using log (x+100). Pearson correlation analysis was first run using Proc Corr to investigate the relationships among all traits measured. The data were then analysed in SAS as split plot experiments pooled over three sites (Steel and Torrie, 1980), and separately for each population using the following model:

$Y = \mu + R + T + RT + C + CT + CR(T) + S + SR + ST + RTS + CS + TCS + SCR(T)$

Where;

Y = observed value;

μ = Overall mean;

R = Replication effect with 3 levels;

T = Treatment effect with 2 levels, i.e., nematodes infested and nematicide treatment;

C = Cycle effect with 4 levels, i.e., C_0, C_1S, C_1R and C_2R;

S = Site effect with 3 levels, i.e., Namulonge, Bufulubi and Kabanyolo.

The error terms were five namely:

Error A = RT = Replication interaction with treatment effect - used to test significance of treatments;

Error B = CR(T) = Cycle interaction with replication effects nested in treatment – used to test significance of cycles, and treatment x cycles interaction;

Error C = SR = Site interaction with replication effect – used to test significance of sites;

Error D = RTS = Replication x Treatment x Site interaction – used to test significance of sites x treatment interaction;

Error E = SCR(T) = Site x Cycle x Replication nested in treatment interaction – used to test significance of sites x cycle interaction, and sites x cycles x treatment interaction.

Differences between means were compared using least significant differences (LSD) at 0.05 significant level. Response to selection was determined by direct comparison of C_0, C_1 and C_2 in replicated trials. Percentage net gain among the cycles was calculated as: $\frac{\text{Advanced population - Original population}}{\text{Original population}}$ x 100 (Keeling, 1982).

Percentage net gain among treatments was calculated as:

$$\frac{\text{Value in nematicide treated plot - Value in nematode infested plot}}{\text{Value in nematode infested plot}} \text{ x } 100$$

Variance components were estimated using residual maximum likelihood (REML) in Genstat (Payne *et al.* 2009). Each population was analysed for the selected traits but only for data under nematode infestation since the interest was establishing the genetic variance among traits under nematode infestation. A pooled analysis was done for the three sites (treated as blocks) since genotype x environmental interaction was trivial. Blocks and cycles were considered as random.

Broad sense heritability (H^2) was obtained using the formula σ^2_g/ σ^2_p (Dabholkar, 1992). Where σ^2_g is the genotypic variance and σ^2_p, the phenotypic variance. Standard error of broad sense heritability was calculated as suggested by Dickerson (1969) where:
SE (H^2) = $2SE(\sigma^2_g)/(\sigma^2_g + \sigma^2_p + \sigma^2_{we})$; $2SE(\sigma^2_g)$ is the square root of the genetic variance , and σ^2_g , σ^2_p and σ^2_{we} refer to the genetic, between plot, and within plot variance, respectively. The latter two are due to common environmental variance or σ^2_e. Selection differential (S) was calculated by subtracting the population mean (comprising all S_1s) from the mean of the selected S_1s to be advanced, i.e., $S = \mu_{sel2} - \mu_o$ for cycle 2 and $S = \mu_{sel1} - \mu_o$ for cycle 1, where μ_{sel2} is mean of the best 10 selected lines to advance to C_2R, μ_{sel1} is mean of the best 20 selected lines to advance to C_1R, μ_o is the mean of the original S_1 population before selection of the best lines. Observed response to selection (R), also described in this article as net gain, was calculated as: C_2R-C_o and C_1R-C_o for cycle 2 and cycle 1 nematode resistant populations, respectively. Realized heritability (h^2) was calculated as: $h^2 = R/S$, where R = response to selection, and S = selection differential (Falconer, 1981).

6.3 Results

6.3.1 Correlations between traits

Plant height and number of ears per plant had positive and significant ($P < 0.05$) correlations with grain yield (Table 6.3). Root mass had highly significant ($P < 0.001$) and positive correlations with grain yield. However, number of root lesions and the *P. zeae* densities were significantly ($P < 0.01$) and negatively correlated with grain yield. Days to anthesis, days to

silking and anthesis to silking interval had significant ($P < 0.01$) and negative correlations with grain yield. *Meloidogyne* spp. densities were not significantly correlated with grain yield although a negative trend was observed.

Plant height had significant ($P < 0.05$) and positive correlations with ear height, root mass, and number of ears per plant but had negative and significant correlations with days to anthesis, days to silking and *P. zeae* densities (Table 6.3). No significant correlations were observed between plant height and number of root lesions, and between anthesis to silking interval and *Meloidogyne* spp. densities. Relationships of ear height with other variables were similar to those of plant height. However, ear height was not significantly correlated with *P. zeae*. Root mass was significantly ($P < 0.001$) and negatively correlated to number of root lesions, days to silking, anthesis to silking interval, and number of ears per plant. Number of root lesions were significant ($P < 0.05$) and positively correlated with days to silking, anthesis to silking interval, *Meloidogyne* spp. and number of ears per plant. Unexpectedly, *Meloidogyne* spp. densities were significant ($P < 0.01$) and positively correlated with the number of ears per plant. The days to anthesis exhibited significant ($P < 0.001$) and positive correlations with days to silking and *P. zeae* but had a significant ($P < 0.01$) and negative correlation with number of ears per plant. The days to silking had similar relationships as days to anthesis except for the significant ($P < 0.001$) and positive correlation with anthesis to silking interval, and no correlation with number of ears per plant. The *P. zeae* densities were significant ($P < 0.001$) and positively correlated with *Meloidogyne* spp. densities.

Table 6.3: Pearson correlation coefficients between traits across sites and across populations

	Plant height (cm)	Ear height	Root mass	No. of root lesions	Days to anthesis	Days to silking	Anthesis to silking interval	*P. zeae*	*Meloidogyne* spp.	No. of ears per plant
Ear height (cm)	0.837***									
Root mass (g)	0.251***	0.251***								
Number of root lesions	0.016ns	-0.054ns	-0.470***							
Days to anthesis	-0.321***	-0.331***	-0.038ns	0.006ns						
Days to silking	-0.222**	-0.287***	-0.252***	0.201**	0.731***					
Anthesis to silking interval	0.033ns	-0.056ns	-0.339***	0.286***	-0.051ns	0.642***				
Pratylenchus zeae (per 100g frm)	-0.206**	-0.097ns	0.050ns	0.099ns	0.291***	0.233***	0.002ns			
Meloidogyne spp. (per 100g frm)	-0.037ns	0.097ns	-0.0486ns	0.278***	-0.016ns	-0.044ns	-0.067ns	0.574***		
Number of ears per plant	0.190**	0.285***	-0.261***	0.312***	-0.177**	-0.116ns	0.034ns	-0.115ns	0.213**	
Grain yield (t ha^{-1})	0.293***	0.351***	0.263***	-0.221**	-0.376***	-0.507***	-0.324***	-0.308***	-0.053ns	0.471***

*, **, and *** represent significance at P ≤ 0.05, P ≤ 0.01 and P ≤ 0.001, respectively. ns = not significant.

6.3.2 Analysis of variance for the three maize populations under nematode treatments at three sites

For Longe 1, treatment effects were significant ($P < 0.05$) for *P. zeae* densities and grain yield but not significant for plant height, root mass, number of root lesions and *Meloidogyne* spp. densities (Table 6.4). The cycle main effects were significant ($P < 0.05$) for all traits measured. Treatment x cycle interaction had significant ($P < 0.05$) effects for *P. zeae* densities. Site main effects were significant ($P < 0.05$) for plant height, root mass, number of root lesions and grain yield but were not significant for *P. zeae* and *Meloidogyne* spp. densities. Site x treatment interaction exhibited significant ($P < 0.05$) effects for only plant height and grain yield whereas site x cycle interaction was significant ($P < 0.01$) only for grain yield.

For Longe 4, treatment main effects were significant ($P < 0.05$) for *P. zeae* densities and grain yield. The cycle main effects, however, were significant ($P < 0.01$) for all traits measured. Site main effects were significant ($P < 0.05$) for root mass, number of root lesions, *P. zeae* and *Meloidogyne* spp. densities. Site x cycle interaction was significant ($P < 0.01$) for root mass and *Meloidogyne* spp. densities.

For ZM521, treatment effects were significant ($P < 0.05$) for only *P. zeae* and *Meloidogyne* spp. densities. However, cycle main effects were significant ($P < 0.01$) for all the traits except grain yield. Site main effects were significant ($P < 0.05$) for only root mass. However, site x cycle interaction was significant ($P < 0.05$) for number of root lesions and *P. zeae* densities.

Table 6.4: Mean squares for traits for all cycles under nematode infested and nematicide treated conditions at three sites in Uganda

Source	DF	Plant height (cm)	Root mass (g)	No. of root lesions	*P. zeae* (per 100g frm)	*Meloidogyne* spp. (per 100g frm)	Grain yield (t ha^{-1})
Longe 1							
Rep	2	449.8	0.292	0.201	6.575	2.928	0.960
Trt	1	601.5ns	0.515ns	0.104ns	40.130*	2.577ns	21.34*
RepxTrt (Err A)	2	76.9	0.097	0.020	1.010	0.665	0.624
‡Cycle	3	786.7***	0.204***	0.114**	3.921***	2.195**	2.471*
TrtxCycle	3	99.1ns	0.009ns	0.021ns	0.389*	0.278ns	0.211ns
RepxCycle/Trt (Err B)	12	74.3	0.009	0.014	0.095	0.249	0.476
Site	2	4039.9*	0.529*	1.596**	1.512ns	2.846ns	24.85*
SitexRep (Err C)	4	320.7	0.058	0.035	2.481	1.086	3.682
SitexTrt	2	36.8*	0.036ns	0.010ns	0.003ns	0.310ns	10.20**
SitexRepxTrt (Err D)	4	3.1	0.034	0.019	0.623	0.166	0.461
SitexCycle	6	41.4ns	0.008ns	0.018ns	0.324ns	0.701ns	3.434**
SitexTrtxCycle	6	124.1ns	0.009ns	0.019ns	0.124ns	0.325ns	0.636ns
SitexRepxCycle/Trt (Err E)	24	79.8	0.016	0.015	0.309	0.307	0.998
Longe 4							
Rep	2	810.6	0.047	0.044	2.148	3.748	0.195
Trt	1	628.9ns	0.077ns	0.173ns	49.99**	1.560*	8.000ns
RepxTrt (Err A)	2	294.8	0.065	0.041	0.227	0.035	2.122
Cycle	3	834.8**	0.420***	0.183***	4.987***	1.463**	5.681**
TrtxCycle	3	148.6ns	0.011ns	0.022ns	0.267ns	0.181ns	1.757ns
RepxCycle/Trt (Err B)	12	135.8	0.009	0.011	0.463	0.150	0.982
Site	2	977.3ns	1.274**	0.524*	17.51*	8.084**	5.952ns
SitexRep (Err C)	4	647.2	0.072	0.049	1.869	0.460	2.463
SitexTrt	2	249.6ns	0.042ns	0.008ns	2.831ns	0.050ns	6.112ns
SitexRepxTrt (Err D)	4	817.6	0.082	0.049	0.518	0.134	1.507
SitexCycle	6	93.8ns	0.078**	0.035ns	0.242ns	0.502**	0.779ns
SitexTrtxCycle	6	113.1ns	0.020ns	0.012ns	0.333ns	0.150ns	0.717ns
SitexRepxCycle/Trt (Err E)	24	114.8	0.019	0.019	0.362	0.108	0.672
ZM521							
Rep	2	1268.2	0.280	0.136	2.458	1.177	2.317
Trt	1	250.1ns	0.057ns	0.001ns	43.16*	2.943**	20.37ns
RepxTrt (Err A)	2	295.5	0.084	0.057	1.596	0.017	3.640
Cycle	3	2123.7***	0.360***	0.219***	1.658**	1.849**	1.311ns
TrtxCycle	3	33.1ns	0.003ns	0.016ns	0.073ns	0.564ns	1.568ns
RepxCycle/Trt (Err B)	12	43.1	0.022	0.011	0.192	0.280	1.737
Site	2	2889ns	1.803*	0.320ns	6.240ns	4.493ns	9.424ns
SitexRep (Err C)	4	981.8	0.137	0.136	4.020	1.867	3.680
SitexTrt	2	173.5ns	0.028ns	0.006ns	3.229ns	0.227ns	11.78ns
SitexRepxTrt (Err D)	4	358.6	0.135	0.014	0.568	0.394	2.333
SitexCycle	6	18.3ns	0.029ns	0.022*	0.631*	0.291ns	1.941ns
SitexTrtxCycle	6	58.9ns	0.011ns	0.019ns	0.426ns	0.060ns	1.043ns
SitexRepxCycle/Trt (Err E)	24	49.6	0.031	0.009	0.211	0.136	0.814

Rep = Replicate, Trt = Treatment (nematode infested vs nematicide treated), Err = Error. *, **, and *** represent significance at P ≤ 0.05, P ≤ 0.01 and P ≤ 0.001. ns = not significant. ‡Cycles evaluated were: C0, C_1R, C1S and C_2R.

6.3.3 Grain yield of the three maize populations in relation to nematode densities

Longe 1 had significantly higher *P. zeae* densities under nematode infested than nematicide treated plots (Table 6.5). *Meloidogyne* spp. densities were also relatively higher under nematode infested than nematicide treated plots. Grain yield was

significantly higher under nematicide treatment (7.0 t ha^{-1}) than under nematode infested plots (5.9 t ha^{-1}).

Both Longe 4 and ZM521 displayed significantly higher *P. zeae* and *Meloidogyne* spp. densities under nematode infested than nematicide treated plots. Grain yield was relatively higher under nematicide treated plots than nematode infested plots in both Longe 4 and ZM521.

Overall reduction in *P. zeae* and *Meloidogyne* spp. densities following treatment with a nematicide was in the range of 72-78% and 41-44%, respectively in the three maize populations. The subsequent gain in grain yield was in the range of 10-19% for the three maize populations.

Table 6.5: Nematode densities and grain yield under nematode infested and nematicide treated conditions

Treatment	*P. zeae* (per 100 g frm)	*Meloidogyne* spp. (per 100 g frm)	Grain yield (t ha^{-1})
Longe 1			
Nematodes	8.4 (6557)	511	5.9
Nematicides	6.9 (1491)	287	7.0
Mean	**7.7 (4024)**	**399**	**6.5**
$LSD_{(0.05)}$	**1.02**	**ns**	**0.8**
Net gain (%)	**-77**	**-44**	**19**
Longe 4			
Nematodes	8.1 (4501)	5.8 (340)	5.9
Nematicides	6.4 (1257)	5.5 (201)	6.5
Mean	**7.3 (2879)**	**5.7 (271)**	**6.2**
$LSD_{(0.05)}$	**0.5**	**0.2**	**ns**
Net gain (%)	**-72**	**-41**	**10**
ZM521			
Nematodes	8.4 (6203)	6.1 (448)	5.8
Nematicides	6.8 (1383)	5.7 (252)	6.9
Mean	**7.6 (3793)**	**5.9 (350)**	**6.4**
$LSD_{(0.05)}$	**1.3**	**0.1**	**ns**
Net gain (%)	**-78**	**-44**	**19**

Untransformed means are presented in parenthesis. Net gains calculated from untransformed means. ns = not significant at 5% probability level.

6.3.4 Performance of the original and advanced cycles of the three maize populations across sites

In Longe 1, plant height was significantly (P = 0.05) higher in C_2R and C_1S and lowest in C_0 (Table 6.6). In Longe 4, plant height was significantly (P = 0.05) higher in C_2R followed by C_1R and least in both C_0 and C_1S. In ZM521, C_2R had the highest plant height whereas C_1S had the lowest but the plant height did not significantly vary in C_1R and C_0. For Longe 1 population, root mass was significantly (P = 0.05) higher in C_1R and C_2R than in C_0 and C_1S. In Longe 4, root mass was significantly (P = 0.05) higher in C_2R and lowest in both C_0 and C_1S. The ZM521 population had significantly (P = 0.05) higher root mass for C_0, C_1R and C_2R compared to C_1S. The number of root lesions was significantly (P = 0.05) higher in C_0 and C_1S compared to C_2R in Longe 1. In Longe 4, number of root lesions was significantly (P = 0.05) lower in both C_1R and C_2R but higher in C_0 and C_1S. The number of root lesions in ZM521 was also lower in C_1R and C_2R and higher in C_0 and C_1S.

Table 6.6: Means for various traits of original and advanced cycles of the three maize populations across sites in Uganda

Cycle	Plant height (cm)	Root mass (g)	Number of root lesions	*P. zeae* (per 100 g frm)	[m]Melo (per 100 g frm)	Yield (t ha^{-1})
Longe 1						
C_0	175.9	3.3 (18.9)	2.96 (10.1)	7.7 (5161)	6.1(520)	6.4
C_1S	190.0	3.3 (18.6)	3.08 (12.5)	8.2 (5104)	6.2(554)	6.0
C_1R	181.0	3.5 (22.9)	2.95 (9.7)	7.6 (3606)	5.9(338)	6.6
C_2R	188.4	3.6 (25.8)	2.89 (8.5)	7.1 (2226)	5.4(183)	6.8
$LSD_{(0.05)}$	**8.53**	**0.10**	**0.12**	**0.31**	**0.49**	**0.68**
Longe 4						
C_0	173.3	3.3 (17.0)	3.0 (11.6)	7.5 (3256)	5.7(289)	5.5
C_1S	172.2	3.3 (18.5)	3.1 (12.6)	7.7 (4487)	6.0(392)	6.6
C_1R	179.1	3.4 (22.1)	2.9 (8.7)	7.1 (2432)	5.5(224)	6.0
C_2R	187.1	3.6 (29.4)	2.9 (8.3)	6.5 (1339)	5.3(176)	6.7
$LSD_{(0.05)}$	**11.5**	**0.09**	**0.10**	**0.67**	**0.38**	**0.98**
ZM521						
C_0	174.1	3.5 (25.7)	3.1 (12.0)	7.8 (5098)	6.1(467)	6.1
C_1S	155.0	3.3 (19.2)	3.0 (11.2)	7.8 (4048)	6.1(425)	6.3
C_1R	171.0	3.6 (27.4)	2.9 (8.7)	7.6 (3728)	5.8(313)	6.4
C_2R	180.6	3.7 (31.5)	2.8 (7.2)	7.2 (2300)	5.4(194)	6.7
$LSD_{(0.05)}$	**6.5**	**0.15**	**0.10**	**0.43**	**0.52**	**ns**

Values in parentheses are the actual means for data that was transformed. [m]*Meloidogyne* spp.

The *P. zeae* densities were significantly (P = 0.05) higher in C_0 and lowest in C_2R in Longe 1. In Longe 4, C_1S recorded the highest *P. zeae* densities whereas C_2R had its *P. zeae* population three times lower than for C_1S. In ZM521, *P. zeae* densities were significantly (P = 0.05) higher in C_0 and C_1S compared to C_1R and C_2R. The *P. zeae* and Meloidogyne spp. densities reduced in C_2R relative to C_1R in the three maize populations. *Meloidogyne* spp. densities were significantly (P = 0.05) lower in C_2R compared to the rest of the cycles in Longe 1. In Longe 4, *Meloidogyne* spp. densities were much lower in C_2R but highest in C_1S. No statistical differences in *Meloidogyne* spp. densities were observed between C_0 and C_1R although C_1R displayed relatively lower *Meloidogyne* spp. densities. ZM521 similarly had significantly (P = 0.05) lower *Meloidogyne* spp. densities in C_2R but very high densities in C_0 and C_1S.

Grain yield in Longe 1 was significantly (P = 0.05) higher in C_2R and lowest in C_1S. Each cycle of selection improved yield by 0.2 t ha^{-1} in Longe 1. In Longe 4, grain yield was significantly (P = 0.05) higher in C_2R and lowest in C_0. Average increment in grain yield was 0.6 t ha^{-1} per cycle of selection for resistance to nematodes in Longe 4. Although there were no significant differences in grain yield among the cycles in ZM521, C_2R had a relatively higher grain yield compared to the rest of the cycles. Similarly, C_0 registered a relatively lower grain yield compared to the rest of the cycles. Thus, an increment in grain yield of 0.3 t ha^{-1} was achieved per cycle of selection.

6.3.5 Gains from selection

In general, there was net gain in plant height, root mass and grain yield in all the three maize populations following the two cycles of selection for nematode resistance (Table 6.7). The number of root lesions, *P. zeae* and *Meloidogyne* spp. densities reduced in all the populations. Longe 1 registered a net gain in plant height in C_2R relative to C_0 but there was no gain relative to C_1S. In Longe 4 and ZM521, net gain in plant height was higher relative to C_1S than C_0. Net gain in root mass was higher relative to C_1S than to C_0 in Longe 1 and ZM521. However, in Longe 4, the net gain in root mass was greater relative to C_0 than to C_1S. The number of root lesions in C_2R had reduced much more relative to C_1S than to C_0 in Longe 1 and Longe 4. In ZM521, the number of root lesions in C_2R reduced more relative to C_0 than C_1S.

The net reduction in *P. zeae* densities in C_2R relative to C_1S and C_0 was within the same range (56-57%) in Longe 1. In Longe 4, *P. zeae* densities reduced more in C_2R relative to C_1S (70%) than to C_0 (59%). This means that C_1S was more susceptible to nematodes than C_0 in Longe 4, which confirms that the selection pressure applied in the direction of susceptibility clearly increased the frequency of susceptible alleles in C_1S genotypes relative to the original source population, C_0. In ZM521, a 55% reduction in *P. zeae* densities was achieved in C_2R after selection relative to C_0 whereas selection relative to C_1S achieved 43% reduction. For *Meloidogyne* spp. densities, net reductions in C_2R relative to C_1S and C_0 were again in the same range (65-67%) for Longe 1. For Longe 4, *Meloidogyne* spp. densities reduced more (55%) relative to C_1S than to C_0 (39%). In ZM521, selection from C_1S to C_2R resulted in a 54% reduction in *Meloidogyne* spp. densities whereas selection from C_0 to C_2R resulted in a 59% reduction. Net gain in grain yield was more (13%) relative to C_1S than to C_0 (6.3%) in Longe 1. However, in Longe 4, net gain was more (22%) relative to C_0 than to C_1S (1.5%). A similar trend was observed in ZM521 with selection from C_0 to C_2R registering 10% net gain in grain yield whereas selection from C_1S to C_2R registered 6.3% net gain.

Table 6.7: Gains in grain yield and other traits following selection in the three maize populations across sites in Uganda

Cycle	Plant height (cm)		Root mass (g)		No. of root lesions		*P. zeae* (per 100 g frm)		*Meloidogyne* spp. (per 100g frm)		Grain yield (t ha^{-1})	
	†Actual	%	Actual	%	Actual	%	Actual	%	Actual	%	Actual	%
Longe 1												
$C_1R - C_0$	5.1	2.9	4.0	21	-0.4	-4.0	-1555	-30	-182	-35	0.2	3.1
$C_1R - C_1S$	-9.0	-4.7	4.3	23	-2.8	-22	-1498	-29	-216	-39	0.6	10.0
$C_2R - C_1R$	7.4	4.1	2.9	13	-1.2	-12	-1380	-38	-155	-46	0.2	3.0
Net gain $C_2R - C_1S$	**-1.6**	**-0.8**	**7.2**	**39**	**-4.0**	**-32**	**-2878**	**-56**	**-371**	**-67**	**0.8**	**13.3**
Net gain $C_2R - C_0$	**12.5**	**7.1**	**6.9**	**37**	**-1.6**	**-16**	**-2935**	**-57**	**-337**	**-65**	**0.4**	**6.3**
Longe 4												
$C_1R - C_0$	5.8	3.3	5.1	30	-2.9	-25	-824	-25	-65	-22	0.5	0.9
$C_1R - C_1S$	6.9	4.0	3.6	20	-3.9	-31	-2055	-46	-168	-43	-0.6	-0.9
$C_2R - C_1R$	8.0	4.5	7.3	33	-0.4	-4.6	-1093	-45	-48	-21	0.7	12
Net gain $C_2R - C_1S$	**14.9**	**8.7**	**10.9**	**59**	**-4.3**	**-34**	**-3148**	**-70**	**-216**	**-55**	**0.1**	**1.5**
Net gain $C_2R - C_0$	**13.8**	**8.0**	**12.4**	**73**	**-3.3**	**-28**	**-1917**	**-59**	**-113**	**-39**	**1.2**	**22**
ZM521												
$C_1R - C_0$	-3.1	-1.8	1.7	6.6	-3.3	-28	-1370	-27	-154	-33	0.3	5.0
$C_1R - C_1S$	16	10	8.2	43	-2.5	-22	-320	-8.0	-112	-26	0.1	1.6
$C_2R - C_1R$	9.6	5.6	4.1	15	-1.5	-17	-1428	-38	-119	-38	0.3	4.7
Net gain $C_2R - C_1S$	**25.6**	**17**	**12.3**	**64**	**-4.0**	**-36**	**-1748**	**-43**	**-231**	**-54**	**0.4**	**6.3**
Net gain $C_2R - C_0$	**6.5**	**4.0**	**5.8**	**23**	**-4.8**	**-40**	**-2798**	**-55**	**-273**	**-59**	**0.6**	**10**

†'Actual' used to refer to means corresponding to the units of measurement indicated. The actual means have been used to calculate gain per cycle.

6.3.6 Realized heritability

Realised heritability (h^2) was generally lower at cycle 1 compared to cycle 2 for all traits across the three maize populations (Table 6.8).

Table 6.8: Selection differentials, response to selection and realized heritability for grain yield and other traits following two cycles of selection under nematode infestation

		Plant height (cm)	Root weight (g)	No. of root lesions	*P. zeae* (per 100g frm)	*Meloidogyne* spp. (per 100g frm)	Yield (t ha^{-1})
Longe 1							
	μ_o	149.4	20.4	17.0	4668	355	6.5
	μ_{sel1}	213.7	28.0	6.8	2095	120	6.9
	μ_{sel2}	214.4	28.7	6.2	1221	110	7.0
	$\mu_{sel1} - \mu_o$	64.3	7.6	-10.2	-2573	-235	0.4
	$\mu_{sel2} - \mu_o$	65.0	8.3	-10.8	-3447	-245	0.5
	$C_1R - C_o$	5.1	4.0	-0.4	-1555	-182	0.2
	$C_2R - C_o$	12.5	6.9	-1.6	-2935	-337	0.4
	h^2_1	**0.08**	**0.53**	**0.04**	**0.60**	**0.78**	**0.50**
	h^2_2	**0.19**	**0.83**	**0.15**	**0.85**	**-**	**0.80**
Longe 4							
	μ_o	136.9	14.0	16.9	3621	245	5.9
	μ_{sel1}	205.4	30.7	6.4	2050	141	6.5
	μ_{sel2}	206.3	33.4	5.3	697	126	7.4
	$\mu_{sel1} - \mu_o$	68.6	16.7	-10.5	-1571	-104	0.6
	$\mu_{sel2} - \mu_o$	69.5	19.4	-11.6	-2924	-119	1.5
	$C_1R - C_o$	5.8	5.1	-2.9	-824	-65	0.5
	$C_2R - C_o$	13.8	12.4	-3.3	-1917	-113	1.2
	h^2_1	**0.08**	**0.31**	**0.28**	**0.52**	**0.62**	**0.83**
	h^2_2	**0.20**	**0.64**	**0.28**	**0.66**	**0.95**	**0.80**
ZM521							
	μ_o	136.0	22.0	18.1	5154	354	6.3
	μ_{sel1}	198.6	35.4	4.4	2229	129	6.8
	μ_{sel2}	200.3	38.2	4.2	1362	69	7.0
	$\mu_{sel1} - \mu_o$	62.6	13.4	-13.7	-2925	-226	0.5
	$\mu_{sel2} - \mu_o$	64.3	16.2	-13.9	-3792	-285	0.7
	$C_1R - C_o$	-3.1	1.7	-3.3	-1370	-154	0.3
	$C_2R - C_o$	6.5	5.8	-4.8	-2798	-273	0.6
	h^2_1	**-**	**0.13**	**0.24**	**0.47**	**0.68**	**0.60**
	h^2_2	**0.10**	**0.36**	**0.35**	**0.74**	**0.96**	**0.86**

μ_o is the mean of the original population before selection of the best lines. μ_{sel2} is mean of the best 10 selected lines to advance to C_2R. μ_{sel1} is mean of the best 20 selected lines to advance to C_1R. h^2_1 = heritability at cycle 1, h^2_2 = heritability at cycle 2. - represents heritability values not presented because they were unrealistic. $\mu_{sel} - \mu_o$ = Selection differential.

For Longe 1, both plant height and number of root lesions had very low (< 20%) realized heritabilities. The other traits had realized heritabilities of 83%, 85% and 80% for root weight, *P. zeae* and grain yield, respectively at cycle 2. *Meloidogyne* spp. had the highest realized heritability (78%) at cycle 1. For Longe 4, realized heritability for plant height and number of root lesions was < 30% for both cycle 1 and cycle 2. Highest realized heritabilities for Longe 4 were observed for *Meloidogyne* spp. densities and grain yield with 95% and 80% at cycle 2 and 62% and 83% at cycle 1, respectively. For ZM521, realized heritabilities were < 40% for plant height, root weight and number of root lesions for both cycle 1 and cycle 2. The realized heritabilities for *P. zeae* densities, *Meloidogyne* spp. densities and grain yield were 47%, 68% and 60% at cycle 1 and 74%, 96% and 86% at cycle 2, respectively.

6.3.7 Broad sense heritability estimates

The broad sense heritabilities (H^2) were variable across cycles of selection for majority of the traits (Table 6.9). Broad sense heritabilities for plant height exceeded twice their standard errors indicating high genetic variance for plant height for all the three maize populations. The broad sense heritabilities for root weight, number of root lesions, *P. zeae* densities, *Meloidogyne* spp. densities and grain yield did not exceed twice their standard errors, indicating low genetic variance at cycle 0, cycle 1 and cycle 2 for the three maize populations. Nevertheless, broad sense heritabilities for root weight were within the same range for Longe 4 and ZM521 (74%-78%) indicating that genetic variances did not decrease over cycles. Genetic variances were too low, if any, for number of root lesions probably because the common environmental variances (σ^2_e) were equally very low for all the three maize populations. A similar trend was observed for *P. zeae*. *Meloidogyne* spp. densities recorded similar and high broad sense heritabilities (81%-90%) for Longe 1 and Longe 4 across the three cycles. Genetic variances and associated broad sense heritabilities were not recorded for some traits because they were negative and this could probably be attributed to sampling error. Broad sense heritabilities for grain yield were highly variable across cycles for the three maize populations due to variations in genetic variances.

Table 6.9: Estimates of genetic variance, phenotypic variance and broad sense heritability for grain yield and other traits under nematode infestation

Parameter	Plant height			Root weight			Root lesions			*P. zeae*			*Meloidogyne* spp.			Grain yield		
	C_0	C_1R	C_2R	C_0	C_1R	C_2R	C_0	C_1R	C_2R	C_0	C_1R	C_2R	C_0	C_1R	C_2R	C_0	C_1R	C_2R
Longe 1																		
δ^2e	23.5	33.30	140.00	0.02	0.00	0.03	0.01	0.01	0.00	0.26	0.45	0.40	0.10	0.41	0.07	0.00	0.80	0.18
δ^2p	113.1	174.60	515.30	0.09	0.05	0.05	0.08	0.05	0.05	0.72	0.47	0.22	0.95	0.40	0.67	5.23	3.00	1.13
δ^2g	89.6	141.30	375.30	0.07	0.05	0.03	0.07	0.03	0.05	0.47	0.02		0.85		0.61	5.23	2.19	0.96
H^2	0.79	0.81	0.73	0.79	0.99	0.49	0.89	0.70	0.96	0.64	0.05		0.90		0.90		0.73	0.84
$SE(H^2)$	0.08	0.07	0.04	2.99	4.48	3.01	3.27	3.81	4.21	0.94	0.31		0.97		1.15		0.49	0.86
$SE(\delta^2g)$	9.47	11.89	19.37	0.27	0.22	0.16	0.27	0.18	0.23	0.68	0.15		0.92		0.78		1.48	0.98
Longe 4																		
δ^2e	26186	135.80	33.60	0.02	0.02	11.2	9.06	7.21	0.00	0.23	53.89	49.2	0.10	0.04	0.11	0.18	0.74	0.44
δ^2p	354.30	214.40	496.10	0.06	0.07	0.22	0.07	0.02	0.03	0.91	1.15	0.85	0.53	0.83	0.64	0.78	1.68	1.68
δ^2g		78.60	462.50	0.05	0.05				0.02	0.68			0.42	0.79	0.53	0.60	0.94	1.24
H^2		0.37	0.93	0.74	0.75				0.88	0.75			0.81	0.95	0.83	0.77	0.56	0.74
$SE(H^2)$		0.04	0.04	3.39	3.26				5.85	0.91			1.24	1.07	1.15	0.99	0.58	0.66
$SE(\delta^2g)$		8.87	21.51	0.22	0.23				0.15	0.83			0.65	0.89	0.73	0.78	0.97	1.11
ZM521																		
δ^2e	129.20	13.40	63.60	0.04	0.03	0.05	9.05	0.00	0.00	0.53	0.06	0.13	39.0	29.0	0.61	0.16	0.84	0.02
δ^2p	219.20	277.90	131.90	0.16	0.12	0.18	0.04	0.02	0.01	0.51	0.89	0.50	0.42	0.68	0.26	2.68	2.50	0.69
δ^2g	90.00	264.50	68.30	0.12	0.09	0.14		0.01	0.00		0.82	0.37				2.52	1.66	0.67
H^2	0.41	0.95	0.52	0.74	0.78	0.75		0.75	0.43		0.93	0.74				0.94	0.66	0.97
$SE(H^2)$	0.04	0.06	0.06	2.16	2.58	2.04		6.18	8.14		1.03	1.21				0.59	0.51	1.19
$SE(\delta^2g)$	9.49	16.26	8.26	0.35	0.30	0.37		0.12	0.05		0.91	0.61				1.59	1.29	0.82

SE = Standard error. Missing values deliberately left out since they were associated with negative genetic variances

6.4 Discussion

The correlation analysis showed plant height, ear height, root mass and number of ears per plant to have a positive relationship with grain yield. Also, the negative correlations obtained between anthesis to silking interval and grain yield indicate that grain yield can be improved if anthesis to silking interval is reduced. Similar results were reported by Bolaños and Edmeades (1996), Byrne *et al.* (2002), Magorokosho *et al.* (2004) but under drought conditions. Number of root lesions and *P. zeae* densities were negatively correlated with grain yield, which implies that grain yield can be improved if number of root lesions and *P. zeae* densities are kept low or eliminated. Tarte (1971) similarly observed a highly significant negative correlation between numbers of *P. zeae* and grain yield of maize in Panama. *Meloidogyne* spp. densities did not show a significant negative correlation with grain yield probably as a result of the low populations, which were below the damage thresholds, in the presence of high *P. zeae* densities. Jordaan *et al.* (1989) similarly recorded few *Meloidogyne* spp. in the presence of high *P. zeae* populations in maize. The significant and positive correlation between *Meloidogyne* spp. densities and the number of ears per plant reflects the host ability to support these nematodes (Seinhorst, 1966).

Generally, nematode densities decreased, and grain yield significantly improved under nematicide treated plots than when plots were left under nematode infestation in the three maize populations. Keetch (1989), Bridge (1994), and Norton and Nyvall (1999) similarly reported reduced maize yields under nematode infestation. Notably, *P. zeae* densities were much higher than *Meloidogyne* spp. densities in all plots. Therefore, *P. zeae* densities were more correlated with reduction in grain yields than the densities of *Meloidogyne* spp. This was further evidenced by the significant relationship of *P. zeae* density with grain yield and non-significant relationship of *Meloidogyne* spp. density with the latter. Similarly, Butseya *et al.* (2005) and Kagoda *et al.* (2010a) found lower *Meloidogyne* spp. densities compared to *P. zeae* densities on maize roots in Uganda. Similar observations were made by Jordaan *et al.* (1989) in South Africa.

The traits which were highly correlated with resistance to nematodes in the current study, such as plant height, days to anthesis, days to silking and grain yield, were improved following the two cycles of S_1 family selection in the three maize populations. However, there were some differences in response to nematodes in the different populations leading to variations in net gain for nematode resistance. For example, Longe 4 showed more improvement in plant height after two cycles of recurrent selection compared to ZM521. Severe nematode infestation has been reported to result in shorter plants (Kimenju *et al.*, 1998; Patel *et al.*, 2002; Luc *et al.*, 2005). Although C_1S and C_0 had shorter plant height

across populations compared to C_1R and C_2R, this was not true in Longe 1 where C_1S had plant height as high as that of C_2R. Zsuzsanna *et al.* (2002) reported that plant and ear height depend on both the environment and the genetic background of the cultivars. Probably the plant height in C_1S of Longe 1 was not reduced following divergent selection because of a genetic background favouring taller plants compared to ZM521 and Longe 4. With every cycle of selection, gain in root mass was higher in Longe 4 compared to Longe 1 and ZM521. Among the cycles, root mass was generally lower in C_1S and highest in both C_1R and C_2R. Smaller root systems are characteristic of nematode injury (McSorley, 1998), which was probably the case in C_1S. Kimenju *et al*. (1998) also observed nematodes to cause significant reductions in root mass of maize OPVs and hybrids.

Number of root lesions indicates level of *P. zeae* damage on the maize roots. Root lesions result from collapse of cell walls causing cavities and tunnels as a result of feeding by root lesion nematodes, such as *P. zeae*, on the cytoplasm of cortical cells (De Waele and Elsen, 2002). Susceptible genotypes are expected to have a large number of root lesions, and this was the case in C_0 and C_1S for all the three maize populations. Net reduction in number of root lesions was, however, higher in ZM521 and lowest in Longe 1. Therefore, *P. zeae* densities were higher in maize populations with greater number of root lesions especially C_0 followed by C_1S in most of the populations. Such populations (C_0 and C_1S) are expected to have low grain yields since the reduction of root tissues reduces the uptake of water and nutrients by the plant (De Waele and Elsen, 2002). A low net reduction from C_0 to C_2R in *P. zeae* was, however, observed in Longe 4 compared to the net reductions in Longe 1 and ZM521. This indicates that Longe 1 and ZM521 responded more to selection for resistance to *P.* zeae than Longe 4. Longe 1 has previously been reported to support few *P. zeae* densities (Kagoda *et al.*, 2010a). Tolerance to *P. zeae* in rice has been linked to drought avoidance strategies, such as deep roots (Plowright *et al.*, 1990), which might also be true for maize. According to Kathiresan and Mehta (2002), *P. zeae* resistance is characterized by penetration of fewer *P. zeae* into the root tissue, delayed egg laying and nematode reproduction, less root necrosis and cell wall thickening around the parasitic zone.

The significantly lower *Meloidogyne* spp. densities in C_2R for all the three populations indicates that this cycle had a higher concentration of *Meloidogyne* spp. resistant genes following recurrent selection. In tomato, the M_i gene is an example of highly expressed resistance that prevents all but trace amounts of root-knot nematode reproduction, resulting in final nematode densities consistently much lower than initial densities (Roberts and May, 1986). Longe 4 generally had lower net reduction in *Meloidogyne* spp. densities whereas Longe 1 had the highest net reduction. Therefore, Longe 1 is more resistant to *Meloidogyne*

spp. followed by ZM521 and lastly Longe 4. However, Longe 4 recorded greater increase in grain yield due to recurrent selection than Longe 1, and ZM521 yielded lowest.

The C_0 and C_1S generally had the lowest grain yields among the cycles. This was a result of these populations having the highest densities of *P. zeae* and *Meloidogyne* spp. Following selection for resistance to plant-parasitic nematodes, a net gain in grain yield of 6.3-22% in the C_2R relative to C_0, was achieved in the maize populations. Relative to C_1S, a net gain in grain yield of 1.5-13% was achieved by C_2R. These yield gains are indicators of the yield loss incurred once nematodes are not controlled in these maize cultivars. Relative to C_0, each cycle of selection for nematode resistance improved grain yield by 0.2-0.6 t ha^{-1} in all the maize populations. Therefore, recurrent selection for nematode resistance is an efficient strategy for improving grain yield in maize cultivars.

Realized heritabilities were very low for plant height and number of root lesions implying that the breeding value was very low for these traits. Though the broad sense heritabilities of these traits were high, they are not very reliable (Falconer, 1981). However, improvement of root weight, reduction of *P. zeae* and *Meloidogyne* spp. densities, and subsequent grain yield enhancement is possible through the S_1 recurrent selection procedure because of the higher heritabilities (> 60%) and greater phenotypic variation observed in the three maize populations. Additive genetic variance was important in improving the heritability of these traits since it is associated with the S_1 progeny selection used in the current study (Weyhrich *et al*., 1998). Root weight, however, had low realized heritability for ZM521. For traits with very low heritabilities, genotypic selection is more efficient though the time needed per cycle of selection would increase. The differences in heritabilities observed among cycles could be attributed to sampling error, genotype x environmental interactions and an underestimation of genetic variance due to linkage disequilibrium as a result of the selection intensity. Wiersma *et al.* (2001) similarly attributed the variations in heritability among cycles to the same conditions.

6.5 Conclusion

The S_1 progeny recurrent selection scheme reduced nematode densities, consequently increasing grain yield in the three maize populations. Notably, each cycle of selection for nematode resistance improved grain yield. The increases in grain yield were, however, more associated with reduced *P. zeae* densities and fewer number of root lesions than reduced *Meloidogyne* spp. densities. Therefore, the study verifies that breeding for nematode resistance using the S_1 recurrent selection procedure can boost grain yield in African maize

populations. The three improved maize populations generated from the current study could be exploited in a breeding programme through interpopulation crosses. However, further selection under nematode infestation may be conducted to determine whether there is a possibility of achieving more gains in grain yield. In the absence of previous studies on recurrent selection, this work forms the baseline for future work towards improvement of maize resistance to nematodes.

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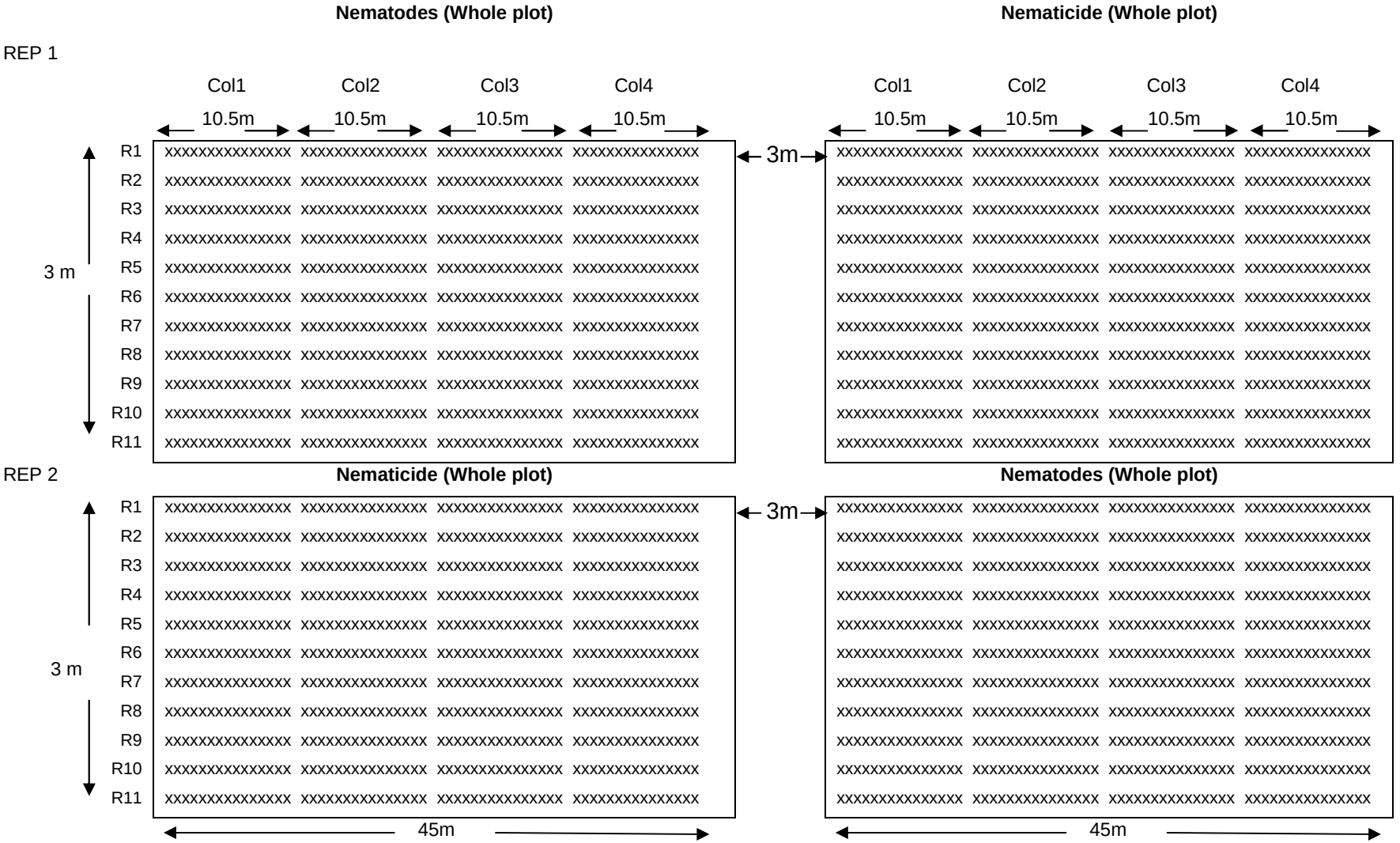
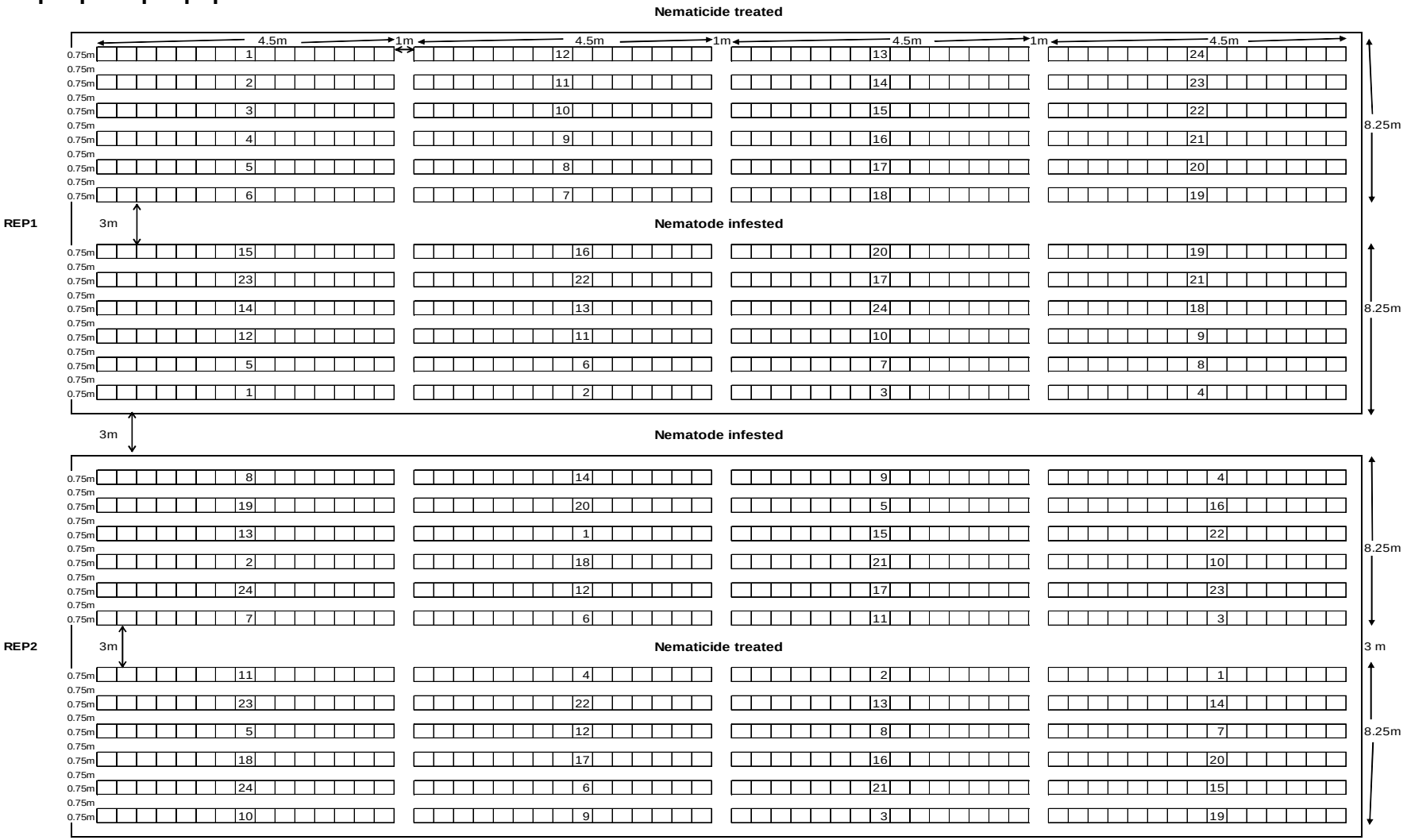
Appendices

Appendix 6.1: Field layout (11 x 4 α-lattice design in split plots) for evaluation of the 44 S_1 families per source populations in the recurrent selection study



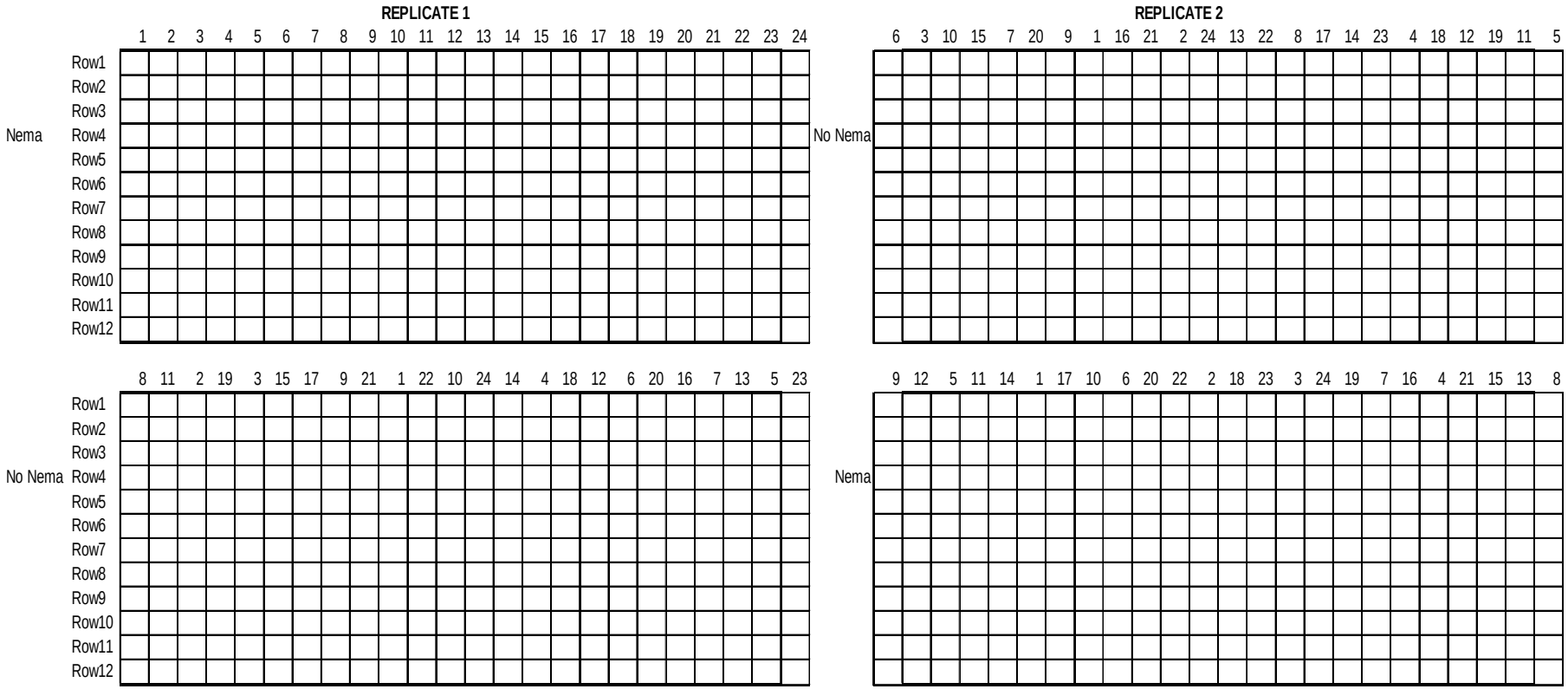
Col = columns; Each of the 11 plot rows (R1 to R11) represents a different family with each family planted in 1 row of 10.5 m length per plot column. Symbol x represents a hill/plant

Appendix 6.2: Field layout for assessment of 20 C_1R lines & 4 checks for resistance to nematodes, arranged in a 6 x 4 α-lattice design in split plots per population



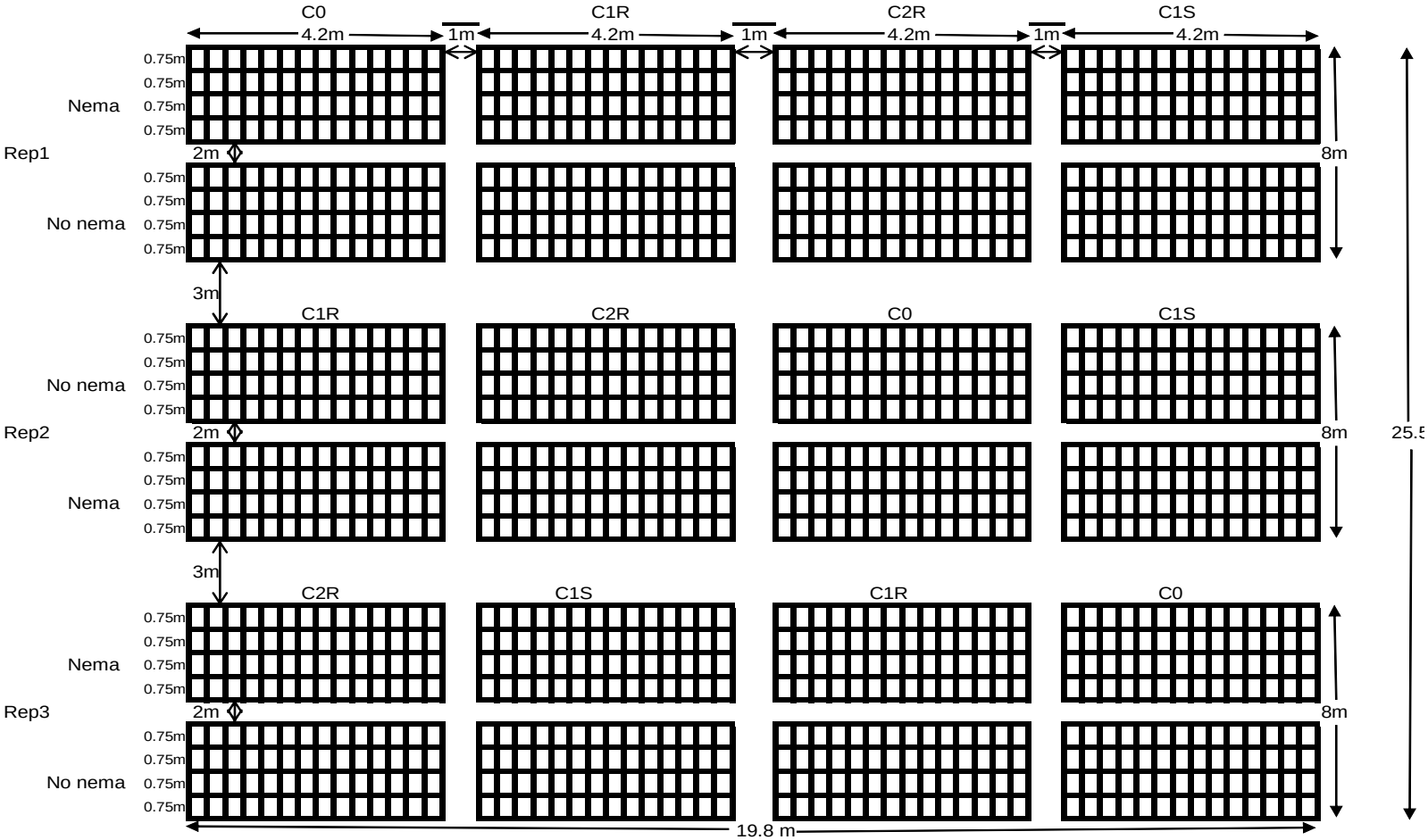
Only layout of a single population is shown. Each T or L-junction represents a hill/plant

Appendix 6.3: Screenhouse split plot design for assessment of 20 C_1R lines & 4 checks for resistance to nematodes per population in the recurrent selection study



Each box, i.e.☐, represents a plastic pot (with 2 500 litres of soil) where the maize seeds were sown. No nema = Pots where soil was sterilised and nematicide applied. Nema = Pots inoculated with 5 000 *P. zeae* or *Meloidogyne* spp. 1 to 24 are arbitrary values for the 24 lines evaluated

Appendix 6.4: Field layout of the multilocation trial, split plot design in the recurrent selection study



Nema = Nematode infested plots; No Nema = Nematicide treated plots. Each T or L-junction represents a hill/plant

CHAPTER SEVEN

General overview

7.1 Introduction

This chapter is a summary of the findings obtained in the entire study. The chapter also summarizes the recommendations and suggests the way forward in developing nematode resistant maize cultivars. The major objective of the study was to assess farmers' awareness of nematodes, the related damage and farmers' desirable attributes in maize, coupled with understanding the genetics of nematode resistance in maize, and yield losses associated with nematode damage. Emphasis was placed on *Pratylenchus zeae* and *Meloidogyne* spp. These are the key nematodes of maize in Uganda and possibly in other maize growing countries in the tropics.

The specific objectives of the study were to:

i. Assess farmers' awareness of maize nematodes, other maize production constraints, and desirable agronomic traits;
ii. Assess the efficiency of sterile carrot discs for mass culturing of *Pratylenchus zeae;*
iii. Characterise the inheritance of nematode resistance in maize, through:
    - Estimating the general combining ability (GCA) of various parents, and the specific combining ability (SCA) of a parent in a cross with another parent;
    - Determining the contribution of cytoplasmic effects to inheritance of resistance to nematodes;
    - Estimating the genetic effects of nematode resistance in maize;
iv. Determine the level of nematode resistance among F_1 hybrids and estimate grain yield, heterosis and yield losses associated with maize hybrids under nematode infestation.
v. Compare the gains in nematode resistance and the resulting grain yield obtained following two cycles of recurrent selection in three tropical maize populations

7.2 Summary of the major findings

7.2.1 Awareness of plant-parasitic nematodes and preferred maize cultivars among smallholder farmers

A participatory rural appraisal was conducted covering 120 households from two districts to assess farmers' awareness of nematodes, and to determine the preferred traits in new maize germplasm. The appraisal established that:

- Only a few farmers (18.5%) are familiar with nematodes and the related damage on maize in Uganda.
- *Pratylenchus zeae* occurred at generally higher frequencies than *Meloidogyne* spp. in nematode susceptible cultivars.
- The landraces and the cultivar Longe 5 supported high nematode populations compared to cv. Longe 1. Farmers also reported low yields for Longe 5 when compared to the rest of the cultivars they grow.
- Farmers' most preferred traits were pest and disease resistance, high grain palatability, long storage duration and large kernels.

7.2.2 Monoxenic culture of *P. zeae* on carrot discs

Twenty live nematodes were transferred to the margins of each of the 40 sterile carrot discs contained in 3.5 cm diameter sterile glass Petri dishes. All cultures were maintained in the dark at 25 ± 1℃.

- The study revealed higher reproduction rates of *P. zeae* on carrot discs compared to excised maize roots. Each *P. zeae* inoculated on the carrot discs reproduced 5,090 times after three months of incubation compared to a reproduction rate of 26.4 on excised maize roots.
- The study indicated that sterile carrot discs are a more productive medium for culturing *P. zeae* than excised maize roots, and is therefore the recommended practice.

7.2.3 Combining ability and genetic effects for nematode resistance in maize

A 6 x 6 full diallel was conducted to study the genetics of nematode resistance in maize. The 30 F_1 hybrids were evaluated in an 8 x 4 alpha-lattice design in split plots at three locations in Uganda.

Combining ability effects

- General combining ability contributed most of the phenotypic variance (72-93%) in reduction of *P. zeae* and *Meloidogyne* densities, and the increase in root mass. The SCA contributed most of the phenotypic variance (43-58%) in plant height and grain yield under nematode infestation.
- Parents CML444, CML312 and CML395 enhanced grain yield with positive GCA effects under both nematode infested and nematicide treated conditions. Parents MP709 and CML206 were the best general combiners associated with *P. zeae* resistance.
- The SCA effects for grain yield were positive and significant for 11 hybrids under nematode infestation. However, hybrids which showed the best SCA effects for grain

yield were MP709/CML312, 5057/CML395, 5057/CML312, CML206/CML312, CML444/CML312 and CML395/CML312.

- Hybrids MP709/CML444 and MP709/CML395 had negative significant reciprocal effects for grain yield resulting from the negative maternal effects observed in parent MP709 under nematode infestation. Therefore, grain yield was being compromised in the two hybrids of this parent when used as the female under nematode infestation.

Genetic effects

- Overdominance gene action explained the non-additive portion of the genetic variance observed for plant height, grain yield, number of root lesions, *P. zeae* and *Meloidogyne* spp. densities.
- For plant height and grain yield, the dominance was largely unidirectional specifically in the direction of tall plants with high grain yield.
- Parents MP709, 5057, CML206 and CML444 contributed most of the dominant genes towards *P. zeae* resistance, whereas CML312 and CML395 contributed recessive genes associated with susceptibility to *P. zeae*. Reciprocal effects were, however, recorded for *P. zeae* densities.
- Parents CML312 and CML206 contributed most of the dominant genes for susceptibility and resistance to *Meloidogyne* spp., respectively. Parents MP709 and CML395 had similar frequency of both dominant and recessive genes.
- Parent CML444 contributed dominant genes for high grain yield whereas MP709, CML395 and 5057 had similar frequency of both dominant and recessive genes towards grain yield.

7.2.4 Nematode resistance, grain yield, heterosis and yield losses among the maize hybrids

The 30 F_1 hybrids generated from the diallel cross were evaluated in an 8 x 4 alpha-lattice design in three locations in Uganda for resistance to nematodes, but also in the screenhouse for *P. zeae* resistance. Two checks were included, one susceptible to nematodes and another resistant.

- Based on the reproduction factor, 24 hybrids were *P. zeae* susceptible whereas six hybrids were *P. zeae* resistant under screenhouse conditions.
- Grain yield was higher by about 400 kg ha^{-1} under nematicide treated plots than under nematode infestation. The nematode resistant hybrids exhibited high yields ranging from 5.0 to 8.4 t ha^{-1} compared to 5.0 t ha^{-1} obtained from the best check.

- Highest grain yield loss due to nematodes was 28%, observed in hybrid CML206/CML444, indicating high levels of damage by nematodes.
- Under field conditions, favourable heterosis was recorded in 18 hybrids for *P. zeae*, and in three hybrids for *Meloidogyne* spp.
- Under nematode infestation, only 16 hybrids had higher relative yield compared to the mean of both checks, the best check and the trial mean, whereas it was 20 hybrids under nematicide treated plots. Hybrids CML312/CML206, CML444/CML395, CML395/CML444, CML444/CML312, CML312/CML444, CML395/CML312, CML312/CML395, CML312/5057, CML395/5057, 5057/CML444, 5057/CML206, CML395/MP709, CML444/MP709 had higher relative yield compared to the mean of both checks, the best check and the trial mean, both under nematode infestation and nematicide treatment.
- Overall, the most outstanding hybrids under nematode infestation were CML395/MP709, CML312/5057, CML312/CML206, CML312/CML444, CML395/CML312 and CML312/CML395.

7.2.5 S_1 progeny recurrent selection

Two cycles of S_1 progeny recurrent selection were used to improve the nematode resistance and grain yield of three OPVs (Longe 1, Longe 4 and ZM521).

- Results revealed that grain yield was negatively correlated with the nematode densities.
- The net gains in grain yield following the two cycles of selection were 6.3%, 10% and 22% for Longe 1, ZM521 and Longe 4, respectively. The average net gains in plant height and root mass were 6.3% and 44%, respectively for the three maize populations.
- The *P. zeae* densities reduced by 57%, 59% and 55%, and the *Meloidogyne* spp. densities by 65%, 39% and 59% for Longe 1, Longe 4 and ZM521, respectively, after two cycles of selection.
- Net reductions in the number of root lesions were 16%, 28% and 40% for Longe 1, Longe 4 and ZM521, respectively, after two cycles of selection.
- Realized heritability (h^2) for *P. zeae* and *Meloidogyne* spp. ranged from 66-96% at cycle 2. For grain yield, h^2 ranged from 80-86% at cycle 2. Broad sense heritability (H^2) for grain yield at cycle 2 ranged from 74-97% for the three maize populations.

7.3 Implications and way forward

- There is need to sensitize farmers on the prevalence of nematodes in maize, especially *P. zeae*, and on the potential control options that are available. In addition to breeding

for nematode resistance in maize, farmers' preferred traits need to be integrated into a breeding programme addressing nematode resistance in maize.

- Carrot discs are suitable, cheaper and reliable medium for culturing *P. zeae* and are therefore highly recommended for raising *P. zeae* populations compared to previously existing methods.

- Parents CML444, CML312 and CML395 enhanced grain yield with positive GCA effects under nematode infestation. Inbreds MP709 and CML206 were the best general combiners for nematode resistance and can therefore be bred into existing genotypes with good agronomic traits. Overdominance gene action explained the non-additive portion observed for inheritance of plant height, grain yield, number of root lesions, *P. zeae* and *Meloidogyne* spp. densities under nematode infestation. The ppreponderance of dominant genes and SCA effects would favour pedigree selection and various sib tests to improve grain yield under nematode pressure.

- Hybrids with favourable heterosis for *P. zeae* and grain yield were obtained, and can be utilised in developing high yielding nematode resistant cultivars.

- It was evident that nematode susceptible hybrids are prone to grain yield losses when grown in nematode infested soils. It is therefore important to breed cultivars resistant to the most important nematodes of maize in uganda.

- Gains in nematode resistance and grain yield can be obtained after two cycles of S_1 progeny recurrent selection. However, there is a need to test the advanced cycles for stability over generations. Similarly, further selection under nematode infestation should be conducted to assess the possibility of achieving more gains in grain yield.