HOST-STATUS OF *CUCUMIS MYRIOCARPUS* TO *MELOIDOGYNE INCOGNITA*

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DECLARATION

I hereby declare that the work herein submitted as a mini-dissertation for the degree Master of Science in Agriculture (Plant Protection) is the result of my own investigation. The work by other authors that formed part of literature support has been duly acknowledged by the reference to the authors.

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DEDICATION

To the Lord, God of all mankind, for His steadfast love, and for knowledge and understanding He blessed with during the times of hardships and struggle. To my family and friends, for their support in my academic journey.

ACKNOWLEDGEMENTS

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ABSTRACT

Host-status of wild cucumber (*Cucumis myriocarpus*) to the root-knot nematode (*Meloidogyne incognita*) was evaluated under microplot conditions. The 30-cm plastic diameter pots were placed into holes leaving 10-cm above the soil surface. Pots were filled to the 10-cm mark (soil surface) using 3:1 sand: Hygromix (v/v). Two-week old seedlings of *C. myriocarpus* were transplanted and irrigated with 2 L tapwater every other day.

On the transplanting day, treatments were initiated by inoculating seedlings with 0, 25, 125, 625 and 3 125 juveniles of *M. incognita*. The treatments were arranged in a randomised complete block design, with 10 replications. The experiment was terminated 56 days after initiating the study.

At all levels of inoculation, the reproductive factor (Pf/Pi) was below unity, suggesting that nematodes failed to reproduce on this plant. Gall formation occurred, but the galls did not develop. Nematode had no effect on shoot and fruit weight, but significantly reduced stem diameter.

Results of this study suggested that *C. myriocarpus* was a non-host to *M. incognita*. The failure of the galls to develop suggested that this plant is resistant to *M. incognita*.

CHAPTER 1
INTRODUCTION

Ground fruit of wild cucumber (*Cucumis myriocarpus*) suppressed numbers of the root-knot nematodes under greenhouse, microplot or field conditions (Mashela, 2002; Mphosi, 2004). In the cited studies, this material increased the productivity of tomato and improved soil electrical conductivity, without affecting soil pH.

The efficacy of ground *C. myriocarpus* on nematode suppression and improving of tomato productivity was comparable to that of synthetic nematicides (Khosa, 2005). In bioactivity tests, Muedi (2005) demonstrated that extracts or fractions of *C. myriocarpus* were nematicidal to *M. incognita* and the citrus nematode (*Tylenchulus semipenetrans*) under microplot conditions.

The material did not interact with *Bacillus* species under all conditions where it was tested (Mphosi, 2004). Also, the material did not have any suppressive effect on *rhizobium* species (Shakwane, 2005).

The toxic substance in *C. myriocarpus* fruits had been identified (Jeffery, 1978; Rimington, 1998; Van Oudsthoorn *et al.*, 1997) as cucurbitacin, which occurs as cucumin ($C_{27}H_{40}O_9$) and leptodermis ($C_{27}H_{38}O_8$). Cucurbitacins are believed to be the most toxic substances from plants. The material occurs in fruits, especially in seeds, and in roots (Haynes and Jones, 1976).

Host-status of plants to nematodes is described in three forms: resistant plants, tolerant plant or susceptible plants. Resistant plants do not allow nematodes to reproduce in them (Barker, 1993; Cook, 1991; Trudgill, 1991). Thus, productivity of resistant plants is not affected by nematodes infection. Tolerant plants allow nematodes to reproduce but there is no reduction in yield (Wallace, 1973). On the other hand, susceptible plants allow nematodes to reproduce in them, but are also expressing yield reduction due to nematode damage (Trudgill, 1992).

Meloidogyne species have a wide host range (McKenry and Roberts, 1985). However, the host-status of *C. myriocarpus* on *M. incognita* is not documented. The objective of the study was to assess the host-status of *C. myriocarpus* to *M. incognita* race 1.

CHAPTER 2
LIRERATURE REVIEW

2.1 Introduction

Widespread suspension of synthetic nematicides has increased focus on alternative strategies of managing nematodes. The use of alternative crops with resistance to nematodes is one of the alternatives being evaluated (Ferraz and Brown, 2002). However, the widespread nature of nematodes and their different races, confer challenges to this alternative.

2.2 Susceptible hosts

Susceptible hosts are plants that have the ability to build up populations of nematodes and also suffer damage in terms of yield reduction. Susceptible plants lack resistance or tolerance or both, making them good hosts for nematode reproduction (Trudgill, 1992). These plants respond to the root-knot nematode (*Meloidogyne s*pecies) attack by forming galls on the roots. Root-knot nematodes are known to cause galls on roots of more than 2 000 plant species (Agrios, 1978). Feeding cells induced by the root-knot nematodes termed giant cells, are formed from host root cells during parasitism to sustain the growth and reproduction of the nematodes (Hussey and Grundler, 1998). The giant cells which are ten times the normal size interfere with the development of the root. Damaged roots can hardly transport water and nutrients from the soil to the developing leaves and shoots. Most of the sugars produced through photosynthesis to support normal root growth are diverted to the giant cells to sustain the developing nematode.

Susceptible plants are stunted, have chlorotic leaves and disfigured roots, in some instances, the plant wilts (Walters and Barker, 1994). The most striking effect of nematode infection is

a general reduction in growth. In the field, a patch of poorly growing plants in an otherwise healthy crop is often the first sign of nematode problems. Fruits on infected plants are of poor size, which sometimes fail to ripen (Dropkin, 1980).

2.3 Tolerant hosts

Tolerance is the mechanism by which plants reduce the extent of damage per unit nematode present (Hayward *et al.*, 1993). Tolerant hosts do not reduce levels of infection, a plant may support high populations of nematodes, but suffer relatively little damage in terms of yield reduction. Chinese holly and dwarf Yaupon holly are tolerant to root-knot nematodes (Barker *et al.*, 1979). *Helicotylenchus dihysera* reproduced successfully on white clover with reproduction factor (Pf/Pi) ranging from 7.4 to 3.2 as initial density rose from 10 to 500 nematodes per pot, but there was no significant effect on plant growth, even at the highest initial density of nematodes (Zahid *et al.*, 2001).

Plants are not passive recipient of destruction by nematodes. Plants can repair damage and regenerate tissues through hormone regulation. Wound responses in the cortical tissues of roots infected with soybean cyst nematode (*Heterodera glycines)* increased nodular efficiency in soybeans (Lehman *et al.*, 1971). The increase of root growth relative to shoot growth in potatoes infected with cyst nematode (*H. rostochiensis)* is well documented (Seinhorst and den Ouden, 1971).

2.4 Resistant hosts

Nematode resistance in plants is expressed in terms of inhibition of nematode reproduction (Ferraz and Brown, 2002). Generally, resistance is expressed as a reproduction factor (RF), which is a ratio of final population density (Pf) to initial population density (Pi). Generally, when the RF is less than one, the plant has some form of resistance against the nematode (Hussey and Barker, 1973). Root-knot nematodes were reported not to cause galling on roots of *Illex attenuate* (Foster 2), *I. crenata* (*'Hetzii'*) or *I. cornuta x aquifolium* 'Nellie R. Stevens' (Bernard and Witte, 1987). Nematode resistance in plants can be due to natural resistance based on the genes present in the plant and can be pre- or post-infectional (Ferraz and Brown, 2002).

2.5 Mechanisms of resistance

Initiation of infection of a root occurs in several steps (Bird, 1974; Doncaster, 1971). Probing of hosts, wall perforation and stylet thrusting, ingestion of nutrients and adjacent members of undifferentiated cells in the central cylinder coalesced through the dissolution of their cell walls, combination of mitosis without cytokinesis and further wall breakdown, to bring about the multinucleate condition and the increase in size of a giant cell. Resistance may occur during the initial stages or afterwards. Mechanisms of resistance are briefly reviewed.

2.5.1 Pre-infectional resistance

Pre-infectional resistance is mainly due to the presence of toxic substances to nematodes. The toxic substances are fully expressed in host tissues before infection and do not rise to higher levels in response to the invading microorganisms. Species of marigold depress

populations of lesion and root-knot nematodes species through pre-formed chemical compounds (Motsinger *et al.*, 1977; Richard and DuPree, 1978). The compounds were identified as alpha-terthienyl and bi-thienyl in roots (Veech, 1981). Asparagus also contains nematocidal glycoside (Rohde, 1972). Certain varieties of alfalfa released substances repellent to tulip root nematode (Griffin and Waite, 1971). Among 175 species of plants of the family surveyed for resistance to the lesion nematode (*Pratylenchus penetrans*), resistance in 70 species was closely correlated with the presence of nematicidal compounds (Gommers and voor in't Holt, 1976). In one study, populations of *P. penetrans* were reduced to 99%, 55% and 63% by *Tagetes patula*, *T. erecata*, and *T. minuta*, respectively. In the same study, fallowing resulted in 43% reduction from the initial population, whereas planting a suitable host (wheat) increased the nematode population by 260% (Suatmadji, 1969).

Phenols are pre-formed chemicals in plants that can cause incompatible host-parasite interaction (Rohde, 1972). Phenol accumulation and products of phenolic oxidation have been associated with cellular browning in a variety of plants infected by several plant-parasitic nematode genera (Giebel, 1970). The phenolic compounds may have an effect on nematode behaviour and metabolism. Chlorogenic acid adversely affected nematode coordination (Chang, 1969). Levels of pre-formed phenols in roots have been positively correlated with resistance of certain plant cultivars to nematodes (Cohn, 1974).

2.5.2 Post-infectional resistance

Post-infectional resistance is the ability of a plant to defend itself against nematode parasitism by releasing chemicals present in low levels to higher concentrations in the host

tissues (Ferraz and Brown, 2002). The chemicals are triggered to higher levels by invading microorganisms, where they either inhibit or kill invading pathogens. The triggered chemicals called phytoalexines confer resistance to pathogens (Harborne, 1999).

Phytoalexines are low molecular-weight antimicrobial compounds which are more or less specific for a plant in which they are formed, but are aspecific in their antimicrobial activity. A phytoalexin diffuses from the hypersensitive tissue and prevents the development of the pathogens. Hypersensitive response and rapid accumulation of phytoalexines provide resistance to secondary infections by pathogens (Bailey *et al.*, 1980). A bean resistant to *P. scriberi* synthesizes a compound that inhibits other nematodes, but no other cases have been reported (Rich and Keen, 1975).

2.5.3 Hypersensitivity

Hypersensitivity is referred to as rapid killing of host cells that limits the growth of the pathogen producing a localized necrosis (Wallace, 1973). Hypersensitivity can be in response to facultative fungi, bacteria, viruses as well as nematodes (Klement *et al.*, 1964; Muller, 1959). Multiplication of pathogens is inhibited in many cells in the hypersensitive spot before the cells develop necrosis (Milne, 1966).

Hypersensitive reaction involves necrotic localized host cells and disorganization as well as restricted pathogen development at the infection site. In the case of nematode attack, the hypersensitive reaction may cause immobilization of the nematode and inhibition of nematode development (Al Tait, 1974; Kaplan, 1978; Orion and Cohn, 1975; Thomason *et*

al., 1976;). Hypersensitivity may be invoked at different times following the initiation of a feeding site by a sedentary parasitic nematode. With sedentary parasitic nematodes such as species of *Meloidogyne*, the hypersensitivity may be invoked relatively early (Paulson and Webster, 1972) before appreciable giant cell formation occurs, or later after giant cell formation has already progressed considerably (Cotten and Hayes, 1969; Endo, 1965).

A frequent response to infection is a rapid browning of cells next to the nematodes. In chrysanthemum leaves infected with *Aphelenchoides ritzemabosi*, browning was very rapid in certain varieties in which the nematodes do not reproduce. Chlorogenic acid and isochlorogenic acid were the chief phenolic substrates and their oxidation and polymerization to form brown pigments following penetration of cells by the nematode's stylet, is therefore associated with resistance (Wallace, 1961). Hypersensitive reaction may sometimes be accompanied by formation of wound periderm and an accumulation of a suberin-like material (Van Gundy and Kirkpatrick, 1964).

Reynolds *et al.* (1970) studied the resistance of alfalfa to juveniles of *M. incognita.* It was found that *M. incognita* penetrated a resistant alfalfa readily but returned to the soil after a few days without inciting suitable giant cells. This was due to the hypersensitive reaction of host towards the feeding of sedentary nematodes. Juveniles that induced cell death around them remained immobilized in resistant roots and ultimately starved. But such juveniles, if removed and inoculated in a susceptible host, will invade and ultimately complete their life cycles (Dropkin, 1969). A hypersensitive response consisting of localized cell necrosis at the

infection site is characteristic of a single gene resistance to many plant pathogens including viruses, bacteria, nematodes and fungi (Keen, 1982).

2.5.4 Genetic resistance

Cucumbers have a repulsion mechanism of incompatibility that comes from genes. Haynes and Jones (1976) found that cucumber plants carrying a dominant allele at the bitter (Bi) locus attracted significantly fewer *M. incognita* juveniles to the roots than did the near-isogenic non-bitter (bibi) genotype. The Bi locus permits plants to accumulate cucurbitacins, toxic triterpenoids that are also important in resistance to other plant pests (Dacosta and Jones, 1971).

The most comprehensively characterized gene conferring resistance to the root knot nematode species is M*i* (Williamson *et al.*, 1994 a,b). The M*i* gene is one of the nucleotide binding sites and leucine rich repeats. The mechanism of resistance to nematodes conferred by M*i* appears to involve a hypersensitive response on the part of non-host. Necrotic plant cells are visible around the head of the invading nematode within six hours of inoculation of roots with nematode juveniles (Dropkin, 1969). Hence, as no feeding site is formed, the juvenile fails to develop and then reproduce (Dropkin *et al.*, 1969). In plants such as tomato and beans, multiple single dominant resistance genes occur and they differentially resist root knot nematode populations (Omwega and Roberts, 1992; Roberts *et al.*, 1990).

Other nematode resistance genes, which have been isolated, and characterized, are H*1*, which conferred resistance to potato cyst nematode (*Globodera rostochiensis)* pathotypes Ro_1 and

Ro_4 with potato cultivars worldwide (Brodie *et al.*, 1998; Ross, 1986). The Gpa2 of potato confer resistance to a small set of populations of *G. pallida (*Van der Vossen *et al.*, 2000). The M*i* and Gpa2 are structurally similar to *R* gene that confers resistance to viruses, bacteria, fungi and insects (Van der Biezen and Jones, 1998).

Resistance has been reported against *M. incognita* in cv. Meashon and Tofany (Al Sayed and Abdel-Hameed, 1991), and against *M. incognita* and root rot in cv. Krygula and Picual (Ghoneim *et al.*, 1996). Allegra, a cultivar developed by the University of California, is reported to have resistance to multiple *Meloidogyne* species in the laboratory (McKenry, 1994).

2.6 Views on conducting host-status experiments

When conducting the host-status of plants to nematodes there are important factors to consider, the level of inoculation depending on the type of crop used, the reproduction factor, damage threshold level and the environmental conditions that are favourable to growth and development of nematodes.

2.6.1 Inoculation

Host-status studies can be conducted under greenhouse, microplot or field conditions. In determining resistance of plants to the root-knot nematodes, eggs are often used as inoculum. Hartmann (1976) indicated that it was important that the level of inoculum to use on a given plant be known. If inoculum rate is too low, susceptible plants may have few root galls and

appear to be resistant, whereas when inoculum rate is too high, resistant plants may have root galls and appear susceptible.

2.6.2 Nematode reproduction

The reproduction of nematodes is mostly determined by the reproduction factor (Pf/Pi), where Pf is the final population densities of nematodes and Pi the initial population densities of nematodes. When the reproduction factor is less than 0.1 then the plant is said to be a non-host, when it ranges from 0.1 to 1 it is said to be a poor host, and when it is greater than 1, the plant is said to be a good host of nematodes (Mojtahedi *et al.*, 1998). Numbers of galls are rated per root system. Nematode galls are rated according to the scale: A rating of 1 = no galls; 2 = 1 to 10 galls; 3 = 11 to 100 galls; 4 = more than 100 galls (Hussey and Barker, 1973).

2.6.3 Damage thresholds

The damage threshold is the initial nematode population density above which yield is suppressed. The damage threshold can be estimated from the Seinhorst equation:

$$Y = m + (1 + m)\, Z^{(Pi - T)}$$

where Y is the relative yield, m is an estimate of the relative minimum yield, Z is a constant representing the proportion of host unaffected by one nematode, Pi is the initial nematode density, and T is the damage threshold (Seinhorst, 1965).

Damage threshold densities by a nematode species vary from one crop to another. Damage thresholds of *M. incognita* on cotton cultivars were reported to be in a range of 1 to 10 eggs +

juveniles/500 cm^3 soil (Roberts *et al.*, 1986; Starr *et al.*, 1989). *Tylenchorhynchus claytoni* does not damage tobacco at high densities, but populations greater than 100/500 cm^3 soil before planting cause decline of azales. A field with *Pratylenchus penetrans* at 500 or more/500 cm^3 soil is more damaging to potatoes, whereas 1-5 juveniles of *P. penetrans*/500 cm^3 soil are sufficient to damage daffodils. Tobacco was harmed more by 25-50 *M. incognita*/500 cm^3 soil than 200 juveniles of *P. brachyurus*, but was not sensitive to any level of *T. claytoni* (Murphy *et al.*, 1974).

Estimates of populations of *R. similis* which cause yield loss to banana are variable. In West Africa, 1 000 *R. similis*/100 g roots were considered to cause serious yield loss, wheras 20 000 *R. similis*/100 g roots were required to cause similar loss in Central America (Gowen, 1995). However, Gowen and Queneherve (1990) reported that 2 000 *R. similis*/100 g roots were a potential cause of yield loss in commercial cultivars. Preplant populations of *R. reniformis* below 300 nematodes/250 cm^3 soil damaged pineapple but were not the major factor limiting yield, whereas at densities above 1 000 nematodes/250 cm^3 soil reduced yield (Sipes and Schmitt, 2000).

2.6.4 Environmental factors influencing nematode damage

Soil environment has a major impact on damage caused by nematodes (Wallace, 1973).

Soil type has an effect because it influences nematode movement as well as being a nutrient and water supply to the host. Soil type influences nematode survival during periods of stress and also influences the species composition of nematode communities (Trudgill and Phillips, 1997).

Root-knot nematodes reproduce well in sandy soil than heavier soils, and they are associated with crop damage in sands or sandy patches of a field (Van Gundy, 1985). Reproduction and development of *R. reniformis* are favored by fine textured soils with a relatively high content of silt and/or clay and moderate levels of clay and silt (Robinson *et al.*, 1987). The damage threshold levels are generally lower in sandy than heavier soils.

Climate and soil may influence pathogenicity and population growth. Barker *et al.* (1976) studied two species of *Meloidogyne* on tomato in a mountain site and a coastal site. The soil type in the mountain site was sandy loam, with an average summer temperature of 20.7°C and the coastal site had loamy sand with an average temperature of 4°C higher. At the coastal site, the population threshold for yield loss was 20 juveniles + eggs/500 cm^3 soil, whereas at the mountain site it was 500-1 000 juveniles + eggs/500 cm^3 soil.

Both temperature and the size of the initial population present at the start of the season affect the final outcome. Nardaci and Baeker, 1979 conducted an experiment where nematodes were inoculated on plants at 24°C and 32°C each. Nematodes reproduced more rapidly on higher temperatures, resulting in higher final population densities. However, as the initial population levels increased, the rate of reproduction declined due to competition for available sites and nutrients in the roots.

CHAPTER 3
PATHOGENECITY OF *MELOIDOGYNE INCOGNITA* ON *CUCUMIS MYRIOCARPUS*

3.1 Introduction

Extracts from fruits of wild cucumber (*Cucumis myriocarpus*) have been shown to consistently suppress densities of the root-knot (*Meloidogyne incognita*) nematodes under greenhouse, microplot and field studies (Mashela, 2002; Mashela and Mphosi, 2001; Mphosi, 2004). Both fruits and roots of *C. myriocarpus* are highly toxic (Jeffery, 1978; Rimington, 1998; Van Oudsthoorn *et* al., 1997). (Keetch and Buckely, 1984, citing Martin, 1995) demonstrated that *C. myriocarpus* was a host to *M. javanica* in Malawi. The major root-knot species in Limpopo Province is *M. incognita*. However, the host-status of *C. myriocarpus* to *M. incognita* is not documented. The objective of this study was to assess the host-status of *C. myriocarpus* to *M. incognita*.

3.2 Materials and Methods

The experiment was initiated on 19 February 2004 in microplots at the Horticultural Unit, University of Limpopo, South Africa (23°53'10" S; 29°44'15" E). Sandy soil was steam-pasteurized at 300°C for 45 minutes prior to mixing. The growing medium comprised 3:1 sand: Hygromix (v/v). Pots were inserted into holes (32 cm diameter, 30 cm height) so that only 10 cm remained above the soil surface, and filled with 2.8 L growing mixture. Inter- and intra-row spacing of pots were both 1 m. *Cucumis myriocarpus* seedlings were raised in seedling trays under greenhouse conditions. The greenhouse day and night ambient temperatures averaged 28°C and 15°C, respectively, with maximum temperatures controlled by thermostatically activated fans.

Seedlings at the four-leaf stage were hardened-off for two days prior to transplanting in microplots, which comprised 30 cm diameter pots, containing the previously described growing mixture. Seedlings were transplanted and hand-irrigated with 1L tapwater every other day.

Meloidogyne incognita race 1 was cultured on tomato Floradade (*Lycopersicon esculentum*) seedlings inside the greenhouse. Nematode eggs and juveniles for inoculum were extracted from infected roots of tomato by the blending and maceration method for 30 seconds in 1% NaOCl and then passed through a 150-µm nested on a 45-µm sieve onto 25-µm. Eggs and juveniles were counted under a microscope and then grouped in a geometric series 0, 25, 125, 625, and 3 125 specimen per 10 ml water vial.

Cucumis myriocarpus seedlings were inoculated by pouring initial population densities of nematodes (Pi) from the 10 ml vial into 10 cm deep holes at the base of each stem. Each infestation level was replicated 10 times, and pots were arranged in a randomised complete block design (RCBD). Seedlings were allowed to establish and treatments, 0, 25, 125, 625 and 3125 nematodes, were applied two weeks after transplanting. Seedlings were irrigated three times a week with 1 L tapwater. Folithion was applied to control aphids when necessary, whereas weeds were manually controlled.

At harvest, 56 days after inoculation, fresh fruits, shoots and roots of *C. myriocarpus* were harvested. Shoots were excised at the soil surface. Stem diameter was measured 2 cm from the severed area using a digital vernier caliber model (T 515). Shoots were oven-dried at

60°C for 96 hours and recorded. Pots were emptied and roots were immersed in water to free soil particles prior to weighing. Root galls were rated according to the scale: 1 = no galls; 2 = 1 to 10 galls; 3 = 11 to 100 galls; and 4 = more than 100 galls (Hussey and Barker, 1973).

Nematodes and eggs were extracted from roots in 1% NaOCl using maceration and blending method (Hussey and Barker, 1973), and the materials were rinsed through nested sieves with the opening sizes of 150-µm and 45-µm and captured onto a 25-µm sieve. Juveniles and eggs (Pf) were collected and counted under a light microscope. The study was repeated on 15 October 2004 and harvested on 10 December 2004.

Data were subjected to analysis of variance using the Statistix program to determine the effects of initial population densities of nematodes (Pi) on the final nematode population densities (Pf) and *C. myriocarpus* yield components. Nematode data were transformed using Ln (x+1) prior to analysis in order to homogenize the variances (Gomez and Gomez, 1984). Mean separation for significant treatments was by the least significant difference test at 0.05 probability level. Regardless of whether the mean differences were significant or not, lines of the best fit between variables and Pi were determined to portray the relationship. Reproduction of root-knot nematodes on *C. myriocarpus* was determined by computing the reproduction factor (Pf/Pi).

3.3 Results

Generally, the results indicated that the polynomial relationship had the best fit between certain variables and Pi of *M. incognita*. However, most relationships were not significant at

$P \leq 0.05$. Because this is a host-status study, the relationships that were not significant ($P \leq 0.05$) were also illustrated.

Final nematode numbers (Pf) increased with an increase in Pi levels (Figure 3.1). Nematodes contributed 99% and 98% in total treatment variation (TTV) of final population of nematode (Pf) in Experiment 1 and Experiment 2, respectively.

The reproduction rate of nematodes increased at low Pi levels and decreased at high levels of Pi (Figure 3.2). Nematodes contributed 98% and 80% in total treatment variation (TTV) of Reproduction factor (Pf/Pi) in Experiment 1 and Experiment 2, respectively. Roots of *C. myriocarpus* responded to Pi levels (figure 3.3). Nematodes contributed 99% and 93% in total treatment variation (TTV) of number of galls in Experiment 1 and Experiment 2, respectively (Figure 3.3).

The Pi level had no effect on shoot weight (Figure 3.4). Similarly, there was no relationship between fruit yield of *C. myriocarpus* and nematode numbers (Figure 3.5).

Stem diameters responded to nematode numbers (Figure 3.6). Nematodes contributed 99% and 91% in total treatment variation (TTV) of stem diameter in Experiment 1 and Experiment 2, respectively. Root weight also responded to nematode numbers (Figure 3.7). Nematodes contributed 58% and 62% in TTV of root weight in Experiment 1 and Experiment 2, respectively.

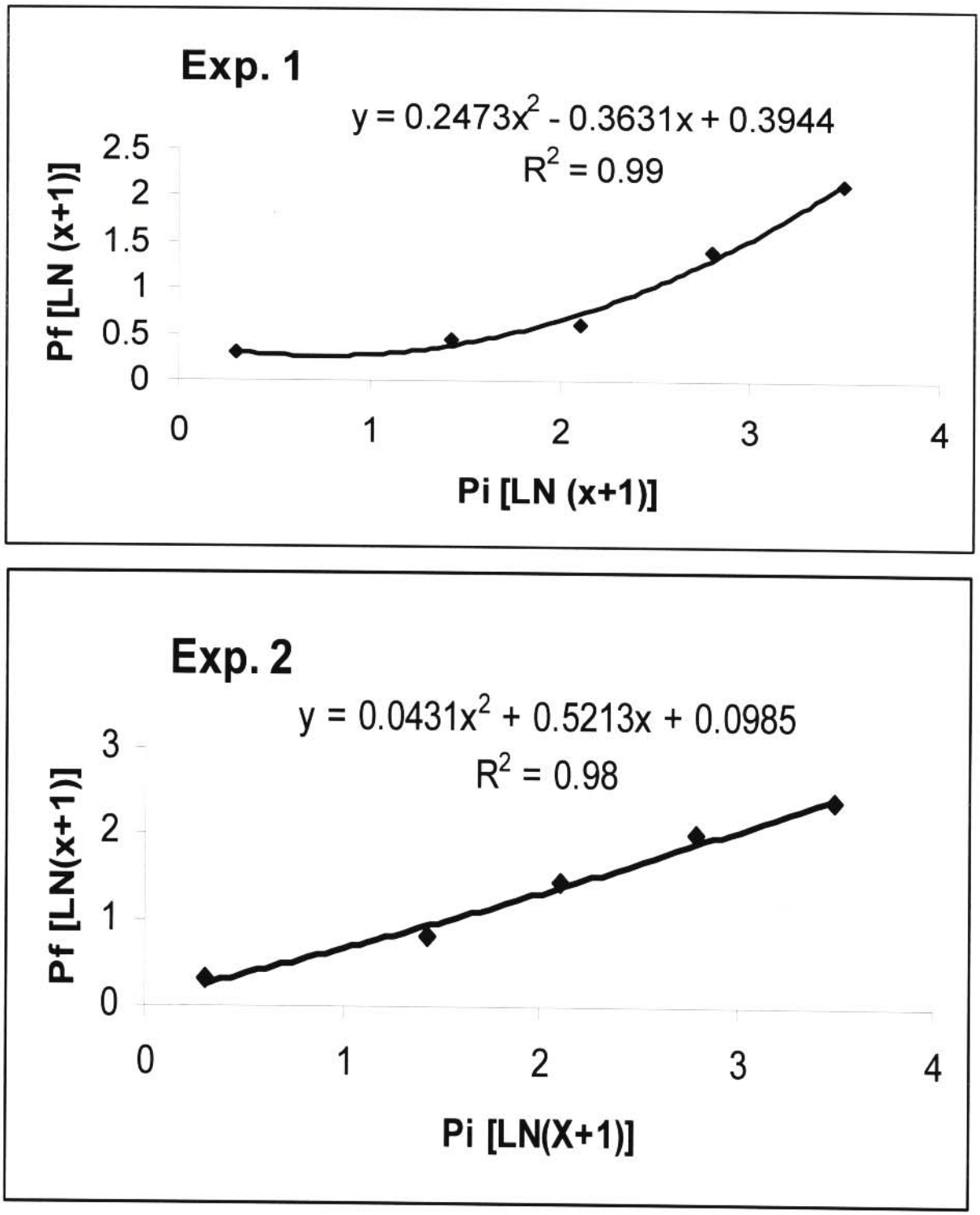


Figure 3.1 Relationship between initial population densities (Pi) of *Meloidogyne incognita* and final population densities (Pf) of *Meloidogyne incognita.*

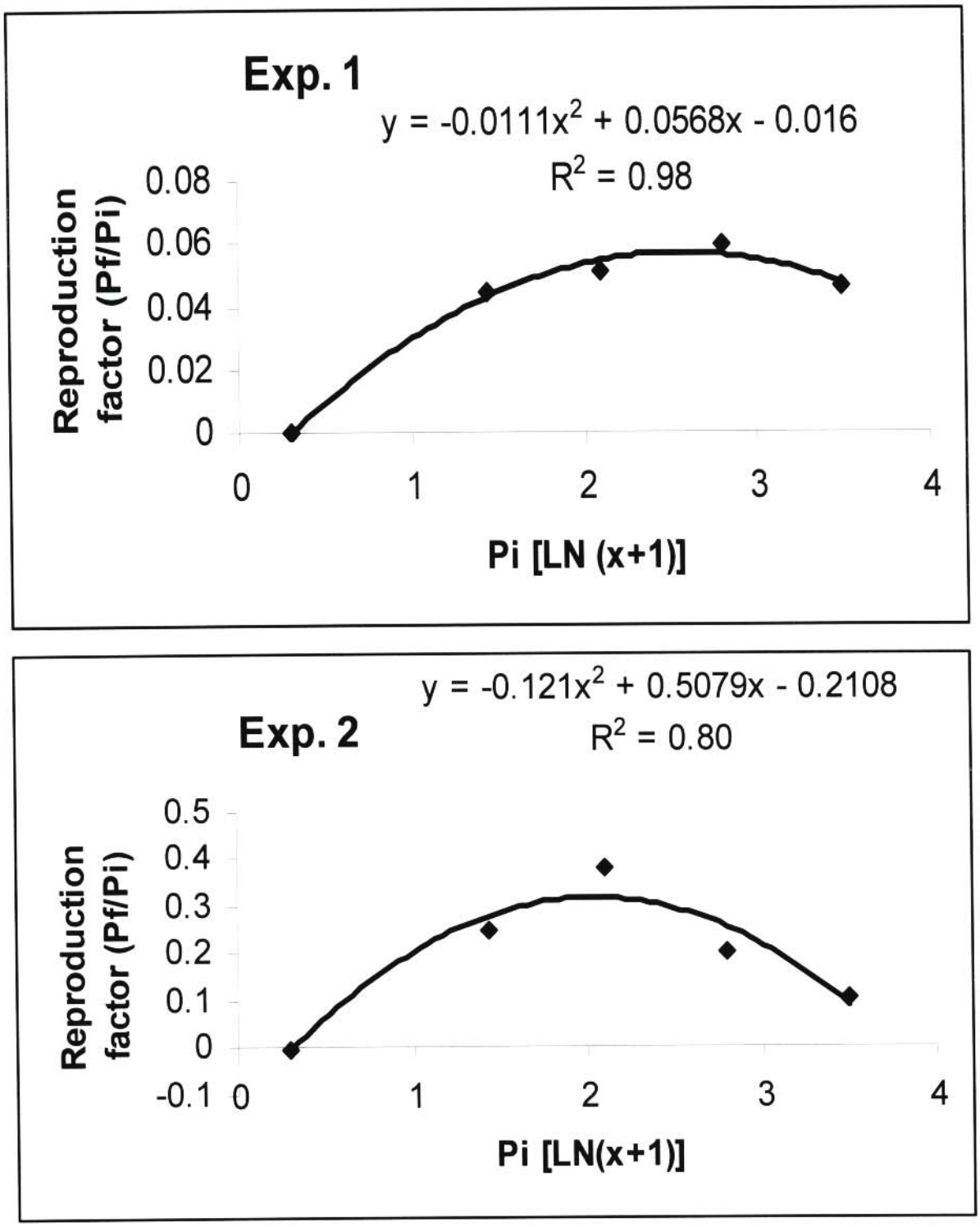


Figure 3.2 Relationship between initial population densities (Pi) of *Meloidogyne incognita* and reproduction rate of *Meloidogyne incognita*.

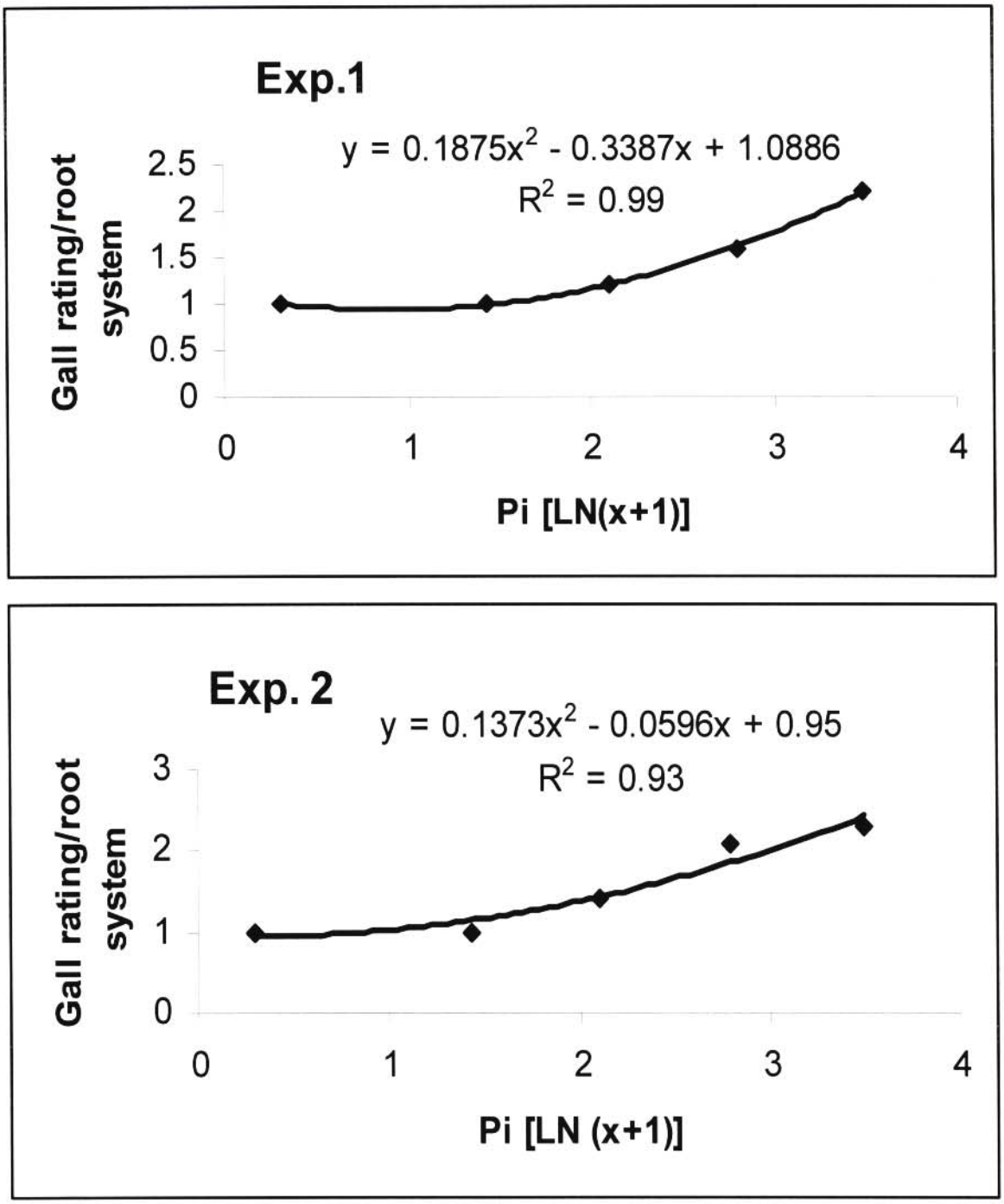


Figure 3.3 Relationship between initial population densities (Pi) of *Meloidogyne incognita* and root gall numbers (1= no galls; 2 = 1-10 galls; 3 = 11-100 galls and 4 = > 100 galls) on the root of *Cucumis myriocarpus* plant.

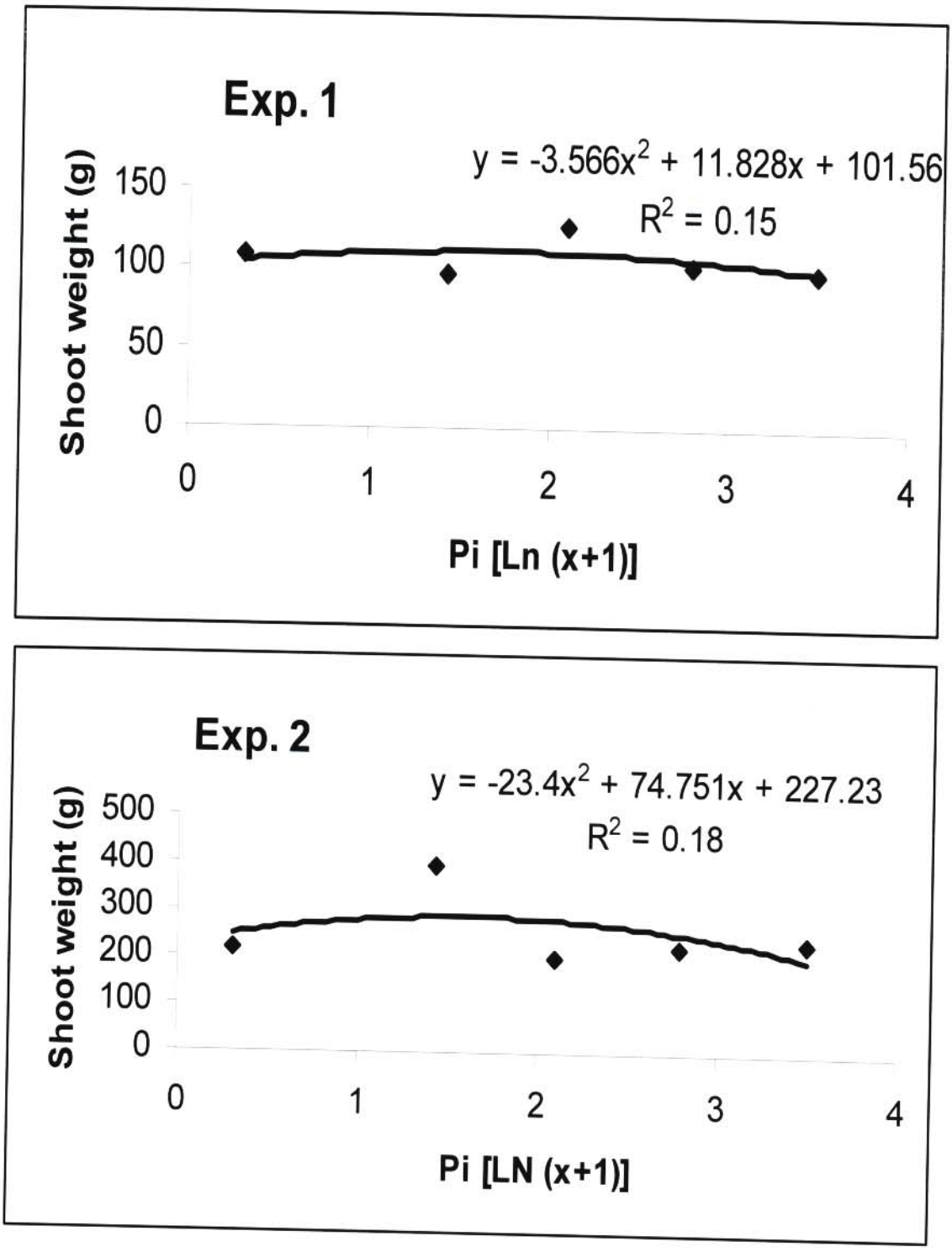


Figure 3.4 Relationship between initial population densities (Pi) of *Meloidogyne incognita* and shoot weight of *Cucumis myriocarpus.*

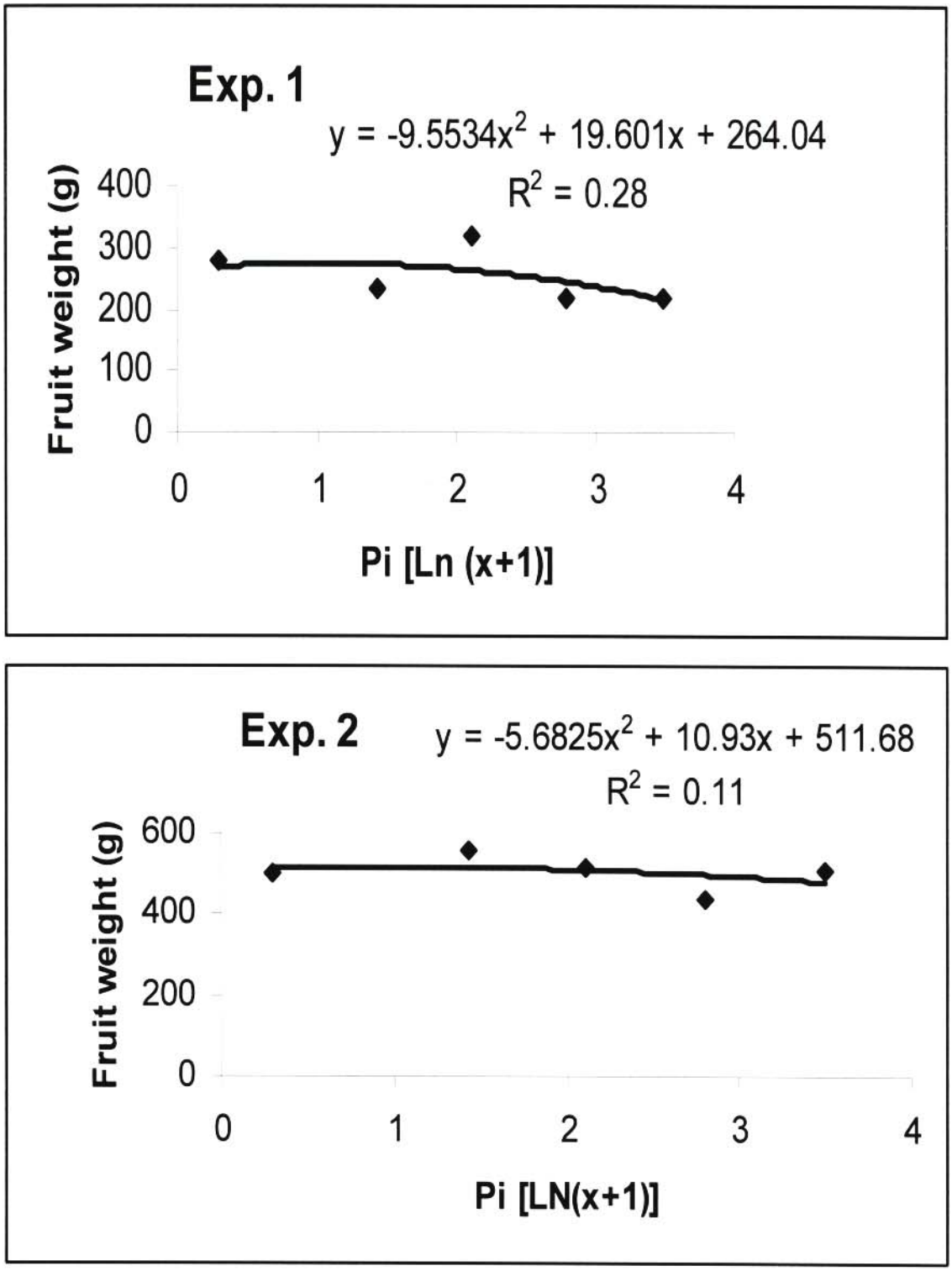
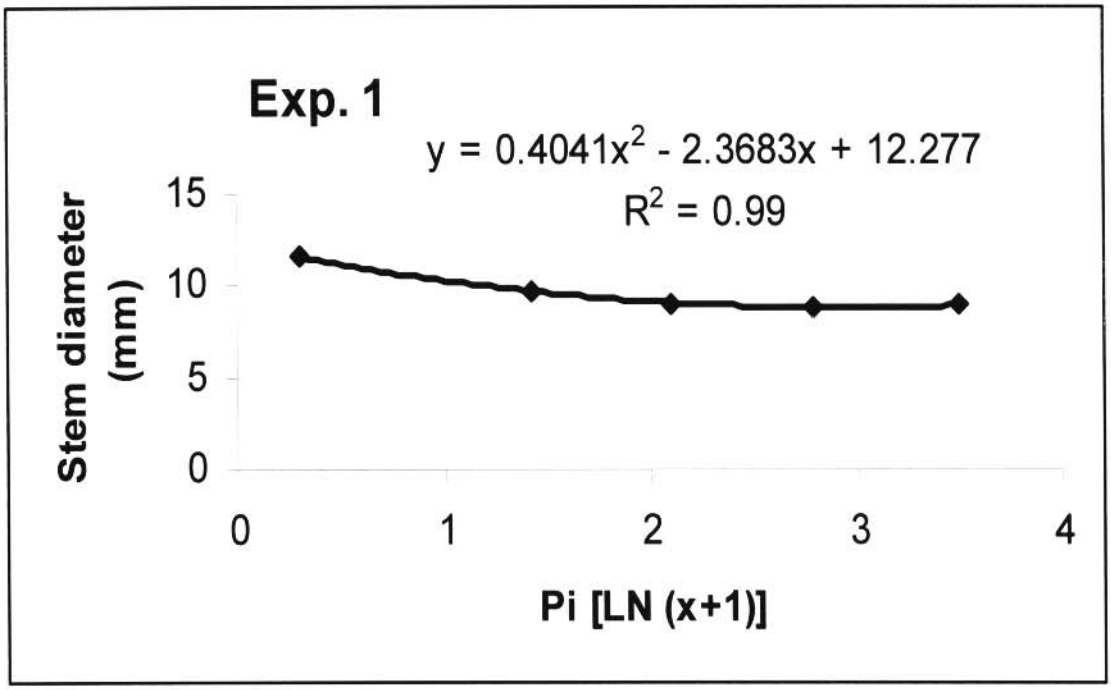


Figure 3.5 Relationship between initial population densities (Pi) of *Meloidogyne incognita* and fruit weight of *Cucumis myriocarpus.*



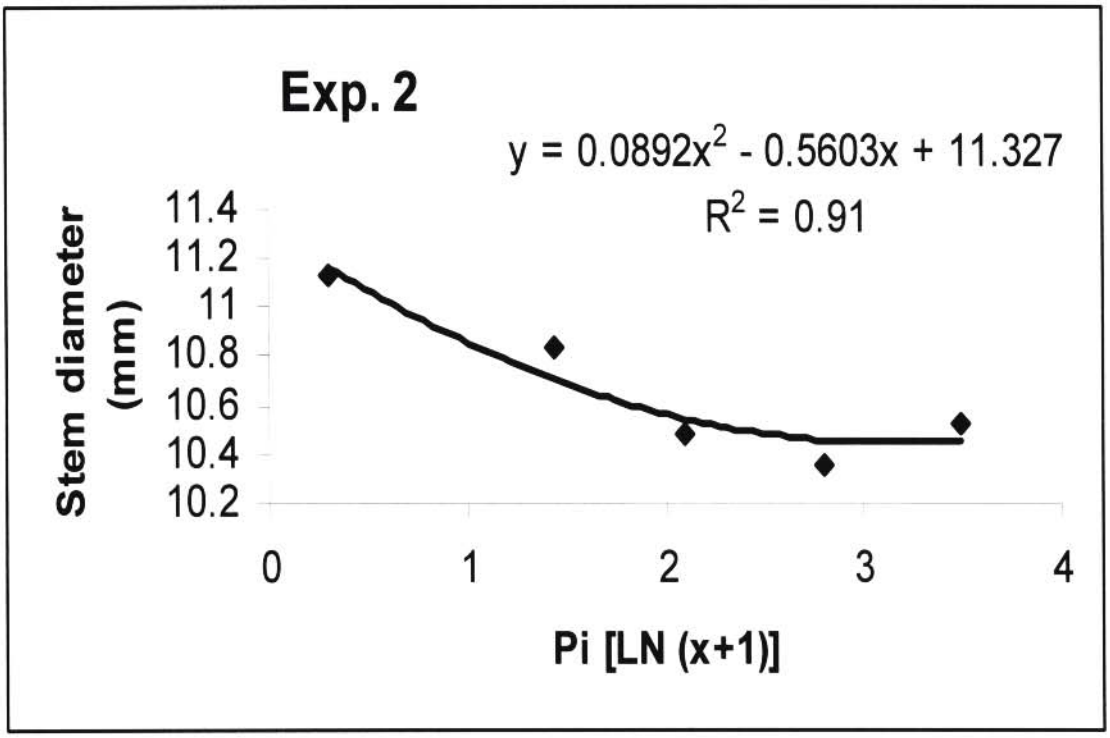


Figure 3.6 Relationship between initial population densities (Pi) of *Meloidogyne incognita* and stem diameter of *Cucumis myriocarpus*.

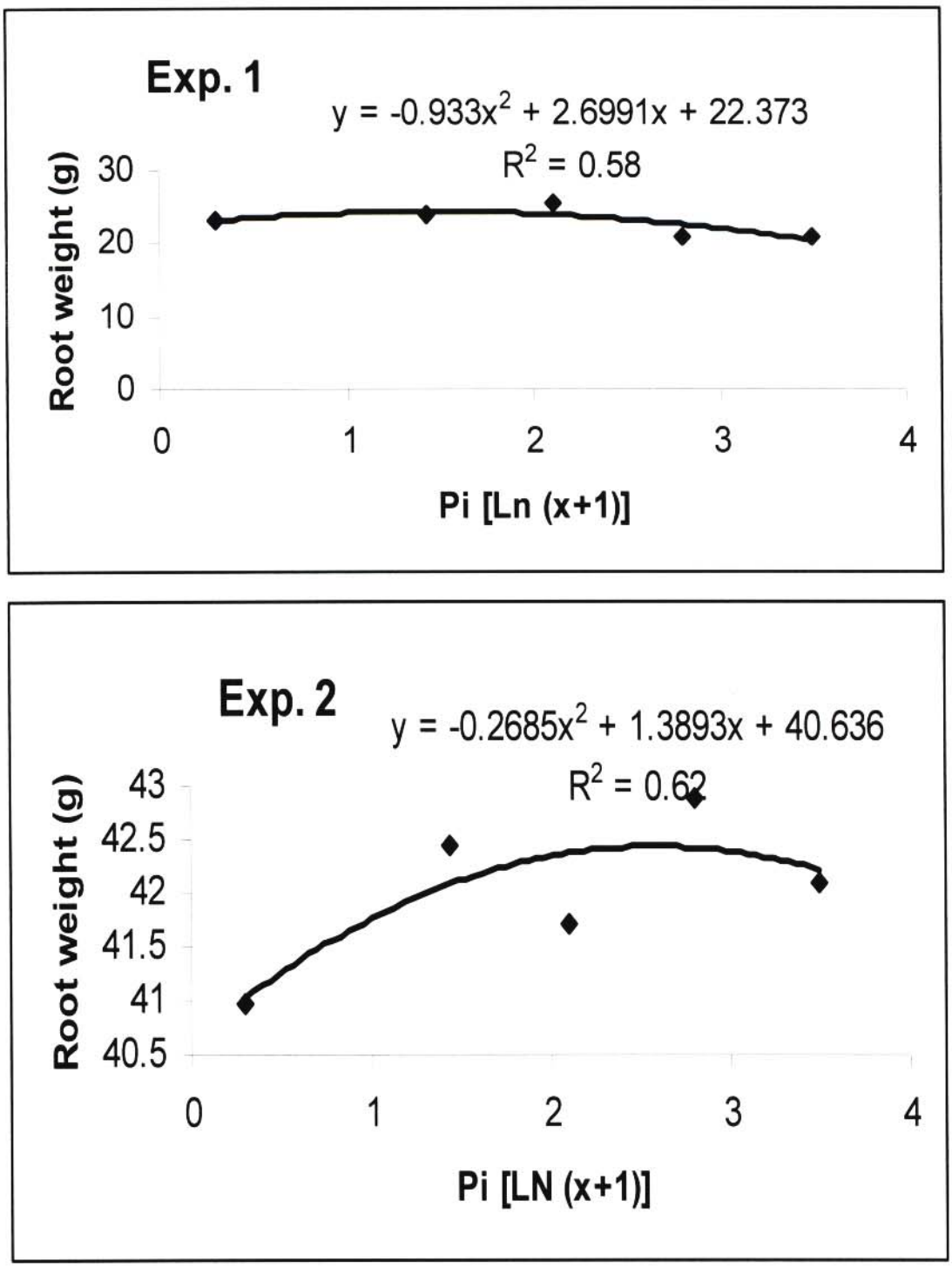


Figure 3.7 Relationship between initial population densities (Pi) of *Meloidogyne incognita* and root weight of *Cucumis myriocarpus*.

3.4 Discussion

The reproductive factor (Pf/Pi) measured was less than one, suggesting that *M. incognita* did not develop and reproduce on *C. myriocarpus* at all levels of Pi. Nematode resistance in plants is expressed in terms of inhibition of nematode reproduction, expressed as a reproduction factor (Ferraz and Brown, 2002). Ten onion cultivars were previously reported to have reproduction factors ranging from 0 to 0.07 to *M. chitwood* race 1 (Mojtahedi *et al.*, 1988). Similarly, *M. chitwoodi* failed to reproduce on three cultivars of carrots (Santo *et al.*, 1988). Generally, a reproduction factor of between 1 and 0 indicates a poor host or non-host to nematodes (Ferris *et al.*, 1993).

Fruits and roots of *C. myriocarpus* contain cucurbitacins, which occur as cucumin ($C_{27}H_{40}O_9$) and leptodermis ($C_{27}H_{38}O_8$) (Jeffery, 1978; Rimington, 1998; Van Oudtshoorn *et al.*, 1997). The cucurbitacin are among the highly toxic compounds in plants. Various studies demonstrated that extracts from ground fruits of *C. myriocarpus* suppress numbers of *M. incognita* on sensitive hosts (Mashela, 2002; Mashela and Mphosi, 2001; Mphosi, 2004). Laboratory bioactivity tests also demonstrated that extracts from fruits of the test plant were biocidal to *M. incognita* and *Tylenchulus semipenetrans* (Muedi, 2005).

Various plants, especially summer cover crops such as certain cultivars of sorghum (*Sorghum bicolor*), cowpea (*Vigna unguiculata*), marigold (*Tagetes species*), castor bean (*Ricinus communis*), velvet bean, and sun hemp demonstrated that they can release chemicals that are suppressive to nematode numbers through root exudation (McSorley and Gallaher, 1991; Roberts, 1993). Species of marigold depress populations of *Pratylenchus* species due

to the nematicidal compounds called alpha-terthienyl and bi-thienyl, that are present in roots (Veech, 1981). The latter compound is capable of inhibiting the hatching of nematode eggs (Siddiqui and Alam, 1988). Penetration and development of *Rotylenchulus reniformis* was reduced within *T. patula* (Caswell *et al.*, 1991), whereas *Meloidogyne* juveniles were unable to fully develop in roots of *T. erecta* (Ploeg and Marris, 1999). Because *C. myriocarpus* also contains high concentration of curcubitacins in roots, it is probable that the observed reduction in the reproductive factor could be explained in terms of the chemicals released through root exudation.

Cucumbers have a repulsion mechanism of incompatibility that comes from genes. Haynes and Jones (1976) found that cucumber plants carrying a dominant allele at the B*i* (bitter) locus attracted significantly fewer *M. incognita* juveniles to the roots than did the near-isogenic bibi (non-bitter) genotype. The B*i* locus permits plants to accumulate cucurbitacins, toxic triterpenoids that are also important in resistance to other plant pests (Dacosta and Jones, 1971). Apparently, the repulsion mechanism was not operative in *C. myriocarpus* because galls, although they failed to develop, were initiated.

Stem diameter of *C. myriocarpus* was sensitive to nematode infection. Generally, it is well documented that infection by *Meloidogyne* species invariably reduces stem diameter of various plants (Mashela, 2002; Mphosi, 2004). Recently, Mafeo (2005) demonstrated that extended irrigation intervals also reduce stem diameter of *C. myriocarpus*. The significance of the reduced stem diameters is to direct more assimilates to roots for growth.

Nematode infection was allowed to a certain degree in this plant, although reproduction of nematodes did not proceed. The partial infection along with the under-developed root galls, may explain the observed increases in root weight. Generally, due to galls, *Meloidogyne* infected roots have higher weight than the uninfected roots (Dropkin, 1980; Mashela, 2002). Redirection of more assimilates to roots may also explain the observed increase in root weight in this study.

CHAPTER 4
SUMMARY AND CONCLUSIONS

Wild cucumber (*Cucumis myriocarpus*) did not support the reproduction of the root-knot nematode (*Meloidogyne incognita* race 1) under microplot conditions. The plant adds to a few plant species that have demonstrated some resistance to *M. incognita*. However, because of the existence of severed rates of *M. incognita*, it is imperative that the host-status of this plant to other races be evaluated.

The various nematode levels had no effect on shoot or fruit yield. Primarily, *C. myriocarpus* will serve as an alternative crop for producing fruits for the extraction of a chemical compound for plant protection. Thus, the observation that the nematode being tested had no effect on fruit yield constitute an important observation.

Because the failed infection of *M. incognita* increased root weight, it is important that the extent of assimilate diversion to roots be determined. Also, the effect of assimilate diversion on the quality and quantity of extracts in fruits may give an indication of whether nematode management in the production of this plant may be necessary or not.

The consistent reduction of the stem diameter in plants infected by *Meloidogyne* species requires additional data to explain its relevance in plant production. In other studies (Mashela, 2002; Mphosi, 2004), reduction in stem diameter was associated with reduction in shoot weight, which agree with the assimilate diversion hypothesis.

Results of this study suggest that *C. myriocarpus* is resistant to *M. incognita* race 1. Thus, the plant has the potential to serve as an alternative crop in low-input production systems of Limpopo Province, where nematodes are a major constraint in crop production.

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APPENDICES

3.1 Analysis of variance for final population densities of nematodes: Experiment 1.

SOURCE	DF	SS	MS	F	P
REP (A)	9	2.65817	0.29535	2.80	0.0135
TRT (B)	4	23.6893	5.92233	56.10	0.0000
A*B	36	3.80026	0.10556		
TOTAL	49	30.1477			

3.2 Analysis of variance for final population densities of nematodes: Experiment 2.

SOURCE	DF	SS	MS	F	P
REP (A)	9	1.40841	0.15649	0.89	0.5431
TRT (B)	4	28.9611	7.24027	41.20	0.0000
A*B	36	6.32661	0.17574		
TOTAL	49	36.6961			

3.3 Analysis of variance for reproduction factor of nematodes: Experiment 1.

SOURCE	DF	SS	MS	F	P
REP (A)	9	0.06196	0.00688	3.47	0.0036
TRT (B)	4	0.02134	0.00534	2.69	0.0467
A*B	36	0.07150	0.00199		
TOTAL	49	0.15480			

3.4 Analysis of variance for reproduction factor of nematodes: Experiment 2.

SOURCE	DF	SS	MS	F	P
REP (A)	9	0.38214	0.04246	1.47	0.1954
TRT (B)	4	0.83371	0.20843	7.23	0.0002
A*B	36	1.03781	0.02883		
TOTAL	49	2.25366			

3.5 Analysis of variance for gall numbers of nematodes: Experiment 1.

SOURCE	DF	SS	MS	F	P
REP (A)	9	1.20000	0.13333	1.09	0.3934
TRT (B)	4	10.4000	2.60000	21.27	0.0000
A*B	36	4.40000	0.12222		
TOTAL	49	16.0000			

3.6 Analysis of variance for gall numbers of nematodes: Experiment 2.

SOURCE	DF	SS	MS	F	P
REP (A)	9	1.12000	0.12444	1.05	0.4238
TRT (B)	4	14.9200	3.73000	31.37	0.0000
A*B	36	4.28000	0.11889		
TOTAL	49	20.3200			

3.7 Analysis of variance for dried shoot weight: Experiment 1.

SOURCE	DF	SS	MS	F	P
REP (A)	9	13612.9	1512.55	1.14	0.3588
TRT (B)	4	6352.63	1588.16	1.14	0.3271
A*B	36	47589.0	1321.92		
TOTAL	49	67554.6			

3.8 Analysis of variance for dried shoot weight: Experiment 2.

SOURCE	DF	SS	MS	F	P
REP (A)	9	861072	95674.6	1.50	0.1838
TRT (B)	4	251642	62910.5	0.99	0.4258
A*B	36	2289340	63592.8		
TOTAL	49	3402053			

3.9 Analysis of variance for fruit weight: Experiment 1

SOURCE	DF	SS	MS	F	P
REP (A)	9	120143	13349.2	0.81	0.6132
TRT (B)	4	79379.3	19844.8	1.20	0.3281
A*B	36	595899	16552.7		
TOTAL	49	795421			

3.10 Analysis of variance for fruit weight: Experiment 2.

SOURCE	DF	SS	MS	F	P
REP (A)	9	941044	104560	1.69	0.1281
TRT (B)	4	75416.8	18854.2	0.30	0.8731
A*B	36	2229971	61943.6		
TOTAL	49	3246432			

3.11 Analysis of variance for root weight: Experiment 1.

SOURCE	DF	SS	MS	F	P
REP (A)	9	380.323	42.2581	0.69	0.7141
TRT (B)	4	159.858	39.9644	0.65	0.6296
A*B	36	2208.71	61.3531		
TOTAL	49	2748.89			

3.12 Analysis of variance for root weight: Experiment 2.

SOURCE	DF	SS	MS	F	P
REP (A)	9	1959.06	217.674	2.56	0.0217
TRT (B)	4	20.7688	5.19220	0.06	0.9928
A*B	36	3058.38	84.9549		
TOTAL	49	5038.21			

3.13 Analysis of variance for stem diameter: Experiment 1.

SOURCE	DF	SS	MS	F	P
REP (A)	9	63.0461	7.00512	1.25	0.2987
TRT (B)	4	53.4797	13.3699	2.38	0.0698
A*B	36	202.244	5.61788		
TOTAL	49	318.769			

3.14 Analysis of variance for stem diameter: Experiment 2.

SOURCE	DF	SS	MS	F	P
REP (A)	9	13.1041	1.45601	1.19	0.3326
TRT (B)	4	3.89155	0.97289	0.79	0.5374
A*B	36	44.1530	1.22647		
TOTAL	49	61.1487			