RESPONSES OF *CAPSICUM FRUTESCENS* CULTIVAR SERRANO AND *CAPSICUM ANNUUM* CULTIVAR CAPISTRANO TO *MELOIDOGYNE INCOGNITA* RACE 2, SALINITY AND GROWTH PERIOD

ANASTASIA N. ALUVILU

[redacted]

DISSERTATION IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE MASTER OF AGRICULTURAL MANAGEMENT (HORTICULTURE)

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UNIVERSITY OF LIMPOPO

SUPERVISOR: PROFESSOR P.W. MASHELA

CO-SUPERVISOR: PROFESSOR M.N. MOKGALONG

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DECLARATION

dissertation hereby submitted to the University of Limpopo, for the degree of Master of Agricultural Management (Horticulture), has not previously been submitted by me or anyone else for a degree at this or any other University. I also declare that this dissertation is my work in both design and execution, and that all materials contained herein had been appropriately acknowledged.

Student: Anastasia N. Aluvilu

16 - 03 - 2010
Date

DEDICATION

This work is dedicated to the loving memories of my late three cousins: Mr John Alphons Pandeni, the former Honourable Minister of Regional, Local Government and Housing, and Rural Development, Ms. Anatolia Mwapopile Muzanima and Dr Elizabeth Hinananye Shangula, all of who passed on during the writing of this dissertation.

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ABSTRACT

The presence of four *Meloidogyne incognita* races limits the use of nematode host-resistance as a management tool in various parts of the world. Since nematode races can only be separated through differential host plants, resistance to one race does not imply automatic resistance to other races. Most vegetable cultivars used in Limpopo Province, South Africa, were bred abroad for resistance against *M. incognita* race 1 and 3, whereas the most dominant race in vegetable-producing areas of Limpopo Province is *M. incognita* race 2. Two separate studies, each with three experiments per pepper cultivar, were conducted to determine the host-status and host-sensitivity of *Capsicum annuum* cv. Serrano and *C. frutescens* cv. Capistrano, to *M. incognita* race 2. Two nematode levels (N_o, N_1) were arranged in a randomised complete block design. At harvest, 56 days after inoculation, *M. incognita* race 2 reproduced on Serrano, but there was no yield reduction, suggesting that this cultivar was tolerant to *M. incognita* race 2. However, cultivar Capistrano did not allow *M. incognita* race 2 to reproduce and did not suffer any yield reduction, suggesting that the cultivar was resistant to this race.

Most salinity studies have had technical limitations in that Ca ions were not included in salt solutions. These ions are invariably required in saline conditions to maintain the integrity of root cell membranes when being subjected to low osmotic potentials. Two separate studies, each with three experiments per cultivar, were conducted to determine the effect of NaCl + $CaCl_2$ salt solutions on the productivity of Serrano and Capistrano cultivars. The two salt levels (S_o, S_1) were arranged in a randomised complete block

design, with 10 replications in Experiment 1 and 8 replications in Experiment 2 and Experiment 3. At harvest, 120 days after the treatment, results suggested that both cultivars were tolerant to NaCl + $CaCl_2$-induced salinities.

Generally, the intensity of a disease is a function of the host, pathogen environment and the exposure time. Thus, studies were performed to examine responses of Serrano and Capistrano to two salinity levels (S_o; S1) and two *M. incognita* levels (N_o; N_1) and three-harvest times (90, 120, 150 days) in a split-split plot arrangement. Treatments and interaction effects were not significant ($P \leq 0.05$) on measured variables in Capistrano, whereas nematode numbers were significantly ($P \leq 0.05$) suppressed under salinity in Serrano and the nematode × time interaction was also significant ($P \leq 0.05$) on various variables measured in this cultivar.

Results in all three studies suggested that Capistrano was both tolerant to salinity and resistant to *M. incognita.* However, Serrano was tolerant to salinity and sensitive to *M. incognita* race 2. Therefore, Serrano is not suitable for use in vegetable crop rotation programmes in Limpopo Province with nematode problems, whereas Capistrano is suitable for use in nematode-infested areas with and without salinity.

CHAPTER 1
GENERAL INTRODUCTION

1.1 Background

Salinity and plant-parasitic nematodes are among the major constraints in crop husbandry in modern agriculture (Ferraz and Brown, 2002; Francois and Maas, 1994; Mashela and Nthangeni, 2002). Worldwide, salinity in irrigated- and dry-land agriculture accounts for 20% and 2% salinity-related crop losses, respectively (FAO, 2005). Global annual crop losses due to plant-parasitic nematode are estimated at 12%: range 6 - 20% (Ferraz and Brown, 2002). Incidentally, linkages between salinity damage and infection by various nematode species in crops have been established (Duncan *et al.*, 1995; Hixson *et al*., 2005; Mashela, 1992). The green revolution approaches previously developed to ameliorate some of the nematode challenges related to crop losses, have been associated with reducing of the ozone layer (Hopwood and Cohen, 2008), which is currently linked to global warming.

Both commercial and subsistence agricultural systems suffer greatly from salinity and nematode problems. Commercial farming systems currently face unprecedented challenges that restrict the use of technologies which were mostly relied upon during the green revolution. For instance, salinity problems were previously managed through elaborate drainage systems (Donahue *et al*., 1990; Doneen, 1975), which removed salts from agricultural land and wantonly dumped them outside of cultivated fields without scant regard of where the salt would eventually end up. Also, the widespread use of fumigant nematicides, with various detrimental environmental effects, resulted in ozone depletion (Hopwood and Cohen, 2008). Since most poor-resource farmers could not afford most of the green revolution technologies, their cultivated lands could still be

considered moderately suitable for agricultural production due to natural balanced systems (Stirling, 1991).

Generally, in most cases salinity and nematode damage in crops have been separately studied. Mashela *et al.* (1992) demonstrated that citrus seedling rootstocks suffered synergistic damage when salinity and the citrus nematode (*Tylenchulus semipenetrans*) occurred simultaneously, whereas cyclic salinity increased the number of nematodes. Recently, Hixson *et al*. (2005) demonstrated that the sting nematode (*Belonolaimus longicaudatus*) and the lance nematode (*Hoplolaimus galeatus*) each inflicted much damage to seashore paspalum in Florida, USA, when nematode infection occurred in the presence of cyclic salinity. Also, cyclic salinity increased population densities of *B. longicaudatus*, *H. galeatus* and *T. semipenetrans* (Hixson *et al.*, 2005; Mashela *et al.*, 1992).

Most salt and nematode studies on crop cultivars focus in selection for salt-tolerance and nematode-resistance, respectively. Mashela *et al*. (1992b) observed that low levels of cyclic salinity eliminated resistance to *T. semipenetrans* in nematode-resistant citrus rootstocks. On the other hand, infection by *T. semipenetrans* eliminated salt-tolerance in salt-tolerant citrus rootstocks (Mashela and Nthangeni, 2002). The disease triangle suggests that the intensity of any disease is a function of host, pest, and environment over time (Agrios, 1997). In most cases, nematode resistant plants are not salt-tolerant and *vice versa*. For instance, all available commercial nematode-resistant citrus rootstocks are highly sensitive to infection by *T. semipenetrans* (Castle *et al*., 1989).

In pepper (*Capsicum* species), resistance to nematodes and tolerance to salinity have been, like in most other crops, separately documented (Ahmad *et al*., 1998; Bethke and Drew, 1992; De Pascale *et al*., 2000; Hare, 1956, 1957; Lee, 2006; Robertson *et al*., 2006). Serrano chilli pepper (*Capsicum frutescens*) and Capistrano green pepper (*C. annuum*) are being reported as being sensitive and resistant to the root-knot nematode (*Meloidogyne incognita),* respectively, in South America (Hartman and Sasser, 1985; Robertson *et al*., 2006; Thomas *et al*., 1995). Generally, *M. incognita* is known to have four races, namely, *M. incognita* race 1, *M. incognita* race 2, *M. incognita* race 3 and *M. incognita* race 4 (Hartman and Sasser, 1985). In the Differential Host Test of North Carolina, all four races reproduced on pepper, although not specified whether it was *C. frutescens* or *C. annuum* (Hartman and Sasser, 1985).Due to the existence of nematode races, it is imperative that empirical evaluation be made to determine which *Capsicum* species is sensitive to *M. incognita* race 2, which is predominant in Limpopo Province, South Africa. Also, most smallholder farmers use borehole water for irrigation, which is inherently salty. Thus, the study of the interaction between salinity and nematode would provide relevant information on the husbandry of pepper, which is becoming one of the major vegetables in Limpopo Province.

1.2 Problem statement

Vegetable farmers in Limpopo Province have limited options of managing nematode and saline problems due to limited availability of nematode-resistant and salt-tolerant cultivars. The researcher proposes to determine the host status of Serrano chilli pepper (*C. frutescens)* and Capistrano green pepper (*C. annuum)* to *M. incognita* race 2 and

salinity, in order to determine whether the two species can be used in crop rotation systems in locations with and without salinity.

1.3 Motivation of the study

Results of the study would provide information as to which *Capsicum* species (*C. frutescens* or *C. annuum)* is suitable for use in areas with and without salinity problems in crop rotation systems against *M. incognita* race 2 in Limpopo Province.

1.4 Aim and objectives

The aim of this study was to characterise the influence of salinity and nematodes on growth and productivity of two pepper cultivars in Limpopo Province, South Africa. Three separate trials were conducted to achieve the following objectives:

Objective 1: To determine the host-status and sensitivity of *C. frutescens* cultivar Serrano and *C. annuum* cultivar Capistrano to *M. incognita* race 2.

Objective 2: To determine the effect of NaCl plus $CaCl_2$ salinity on the productivity of *C. frutescens* cultivar Serrano and *C. annuum* cultivar Capistrano.

Objective 3: To determine the interaction responses of the productivity of *C. frutescens* cultivar Serrano and *C. annuum* cultivar Capistrano to salt, *M. incognita* race 2 and duration of growth.

1.5 Hypotheses

Three hypotheses, each corresponding to the listed specific objectives, were formulated:

Hypothesis 1: *Capsicum frutescens* cultivar Serrano and *C. annuum* cultivar Capistrano are neither host nor sensitive to *M. incognita* race 2.

Hypothesis 2: Sodium chloride plus $CaCl_2$ salinity do not have effects on the productivity of *C.* frutescens cultivar Serrano and *C. annuum* cultivar Capistrano.

Hypothesis 3: Salt, *M. incognita* race 2 and duration of growth do not have interactive effects on the productivity of *C. frutescens* cultivar Serrano and *C. annuum* cultivar Capistrano.

1.6 Format of the dissertation

The Chapter on General Introduction was followed by that on Literature Review, with each objective also constituting a chapter. The sixth chapter provided the Summary and Conclusions of the study.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

In this literature review, the impact of salinity, nematodes and their interactions were reviewed, with emphasis being on the work done in pepper with regard to the main factors studied. Also, existing gaps with respect to the main factors in pepper production were identified. Other crops, where information is not documented with respect to the two factors, were used as models.

2.2 Agricultural salinity

Worldwide, salinity is increasing at alarming rates in agricultural land, particularly in arid and semi-arid regions (Francois and Maas, 1994). In regions with high annual rainfall, salinity is a cyclic problem, with salt accumulating in the rhizosphere during non-rainy seasons and being leached out during rainy seasons (Mashela *et al*., 1992a). The concept of cyclic salinity, coined by Mashela *et al*. (1992a) using the citrus nematode (*Tylenchulus semipenetrans*), provided the first insight on the mechanism through which

salinity increased nematode numbers when compared with untreated control under field studies in Texas, USA (Machmer, 1958).

Salinity is a condition where excessive salt accumulation in the root-zone impedes plant growth (Bohn *et al*., 1985). Generally, salinity is quantified in units of electrical conductivity of soil extract (EC_e), sodium adsorption ratio (SAR) and soil pH (Bohn *et al*., 1985). Non-salinity is the soil condition where $EC_e < 4$ dS/m, SAR < 15 and pH < 8 (Bohn *et al*., 1985; Sposito, 1989). In contrast, salinity is a condition where $EC_e > 4$ dS/m, SAR > 15 and pH > 8.

2.2.1 Impact of salinity type on crops

Three salts associated with agricultural salinity are the chlorides, sulphates and carbonates, with Na and Cl ions being referred to as salinity ions due to their dominance (Bohn *et al*., 1985). Stragonov (1962) and Poljakoff-Meyer (1975) demonstrated that in industrialised countries SO_4 salinity was the most typical and that it was the most damaging to plants and property since it induces acid rain. Curtin *et al.* (1993) reported that Ca deficiencies were most common in plants that were grown in regions with sulphate salinity, since the two ions form non-exchangeable $CaSO_4$ precipitate. However, Bohn *et al*. (1985) demonstrated that crops grew better under SO_4 salinity than Cl salinity when Ca ions were not limiting.

Peynado and Young (1963) documented the severity of salt damage in *Citrus* species on the basis of salt-type. In the study, salt-damage-induced chlorosis was in the order of

$CaCl_2$ > Na_2SO_4 > NaCl in both sand and loam soils, whereas the severity of bronzing was in the order of $CaCl_2$ > NaCl > Na_2SO_4. Overall, NaCl and Na_2SO_4 each reduced *Citrus* growth more than $CaCl_2$, probably due to the damaging effect of Na to both plants and soils. In another study (Epstein, 1961), the reduced effect of $CaCl_2$ in decreasing plant growth has been ascribed to the ameliorating effect of Ca ions on cell membranes. The observations in the salt-type studies agreed with the solubility of the salt types, with that of $CaCl_2$ being 25 470 mols/ m^3, NaCl 6 108 mol/m^3 and Na_2 SO_4 683 mols/m^3 (Doneen, 1975). Apparently, the higher the solubility of a salt, the higher is the damage potential of the related salinity ions to crops.

Effect of NaCl and Na_2SO_4 were compared on the ability of *Pisum sativum* root to synthesise proteins. A direct relationship was demonstrated between salt concentration and the amount of amino acids incorporated into proteins (Poljokoff-Meyer, 1975). In the study, the inability to incorporate amino acids into proteins was greater when plants were grown in Na_2SO_4 compared to NaCl. Plants grown at high salt levels had a decreased ability to incorporate amino acids into proteins even if removed from the salt solution, suggesting that SO_4 and Cl salts might regulate the production of different proteins.

2.2.2 Effect of salinity type on soil

The effect of Na_2CO_3 salinity on plant is mainly through increasing Na hazard in soils (Bohn *et al*., 1985; Sposito, 1989). Bicarbonate ion in soil solution reacts with Ca to form a non-exchangeable $CaCO_3$ precipitate (Bohn *et al*., 1985). Precipitation of $CaCO_3$ reduces the concentration of Ca in soil solution, thus, increasing the sodium adsorption

ratio (SAR), which implied increased exchangeable Na ions in soil solution and an increased Na hazard (Bohn *et al.*, 1985). The increase in soil pH under carbonate salinity may also induce nutrient deficiencies in certain plants (Bohn *et al.*, 1985).

2.2.3 Sources of agricultural salinity

In regions near oceans the primary sources of Na and Cl ions are mainly salt-water intrusion into freshwater resources, rain and plant residues (Graham, 1990). Most agricultural salinity is due to contaminants from fertilizers which could not be successfully removed during the manufacturing processes (Bohn *et al*., 1985). Irrigation with poor quality water, especially in arid and semi-arid regions, remains the main source of agricultural salinity. When crops are irrigated with poor quality water, upon absorption of water through roots and the evaporation of some water from the soil surface, large quantities of salinity ions remain in the rhizosphere, resulting into salinity problems (Bohn *et al*., 1985).

2.3 Salinity-crop interaction

Salinity interacts with plant growth and development at different physiological levels. Reduction in crop growth by salinity had been attributed to excess salinity ions in the soil, imbalance of water and nutrient elements, chlorophyll content and protein metabolism (Munns, 2002; Zhu, 2001).

2.3.1 Yield reduction in crops

Plant growth is an irreversible increase in size and form of plants through cell division and cell enlargement (Fog, 1963; Fosket, 1994). Cell division is genetically regulated during plant development, whereas cell enlargement is a water-uptake-driven expansion of the cell walls (Fosket, 1994). Chartzoulakis and Klapaki (2000) demonstrated that plant growth variables such as plant height, total leaf area and dry shoot mass were

significantly reduced at salinities higher than 25 mM NaCl in pepper (*Capsicum annuum*) cultivar Sonar and cultivar Lamuyo, which were salt-tolerant and salt-sensitive, respectively.

Generally, plants are most susceptible to salt damage during germination and at the seedling stage than at any other stage (James, 1988). Contrary to the observations by James (1988), Yildirim and Güvenc (2006) observed that most salt-tolerant pepper cultivars had improved seed germination and seedling vigour under salt-stress conditions. At salinities up to 50 mM NaCl, delayed germination of pepper seeds was observed, but the delay did not reduce the final germination percentage (Chartzoulakis and Klapaki, 2000). Seedling stage is mainly that stage when the plant is still depended upon the cotyledon leaves for its chlorophyll content. Sensitivity of seedlings to salinity might be caused by the localized high salinity concentration within the effective root-zone of the seedlings, thus, making it more difficult for seedlings to absorb water that moves down the water potential gradients. Effective root-zone is a soil depth range where 80-90% of the roots occur (Bohn *et al*., 1985).

Low water potentials outside roots relative to those inside of roots result in water moving from roots into the soil with immediate suppression of leaf growth (Munns *et al.*, 1995). Increasing the level of soluble salts in soil solutions tended to increase its osmotic pressure, thus, suppressing water and nutrient uptake (Smith *et al.,* 1992). Generally, salt tolerance in leaves is determined by the ability of the plant to accumulate the ions within

compartments of the leaf cells where they will not interfere with parenchyma cells, which are responsible for cell division (Flowers and Yeo, 1989).

A reduction in root elongation due to salinity prevents the ions that move by diffusion towards the root from reaching the shoot in the required quantities (Kafkafi, 1991). Growth reduction under salt stress had been attributed to accumulation of ions to toxic levels in plant tissues and to osmotic stress due to low external osmotic potential (Bohn *et al*., 1985). Cell division, cell enlargement and the production of proteins and nucleic acids are some of the physiological processes that are retarded by high levels of salt with immediate effect (James, 1988).

2.3.2 Responses of chlorophyll content to salinity

Primary by-products of chlorophyll content are soluble sugars (Campbell, 1990). When plants are exposed to salt stress, they adjust osmotically by using a portion of photosynthetic assimilates to increase their osmotic potential so that water continues to enter the cells (Chow *et al.*, 1990). Bunals *et al*. (1991) studied growth and gaseous exchange variable parameters of citrus plants stressed with different salt types, where Cl salt such as NaCl and KCl, progressively reduced chlorophyll content. In the study, the decrease in chlorophyll content coincided with a significant decrease in stomatal conductance and leaf gas exchange.

A decrease in leaf chlorophyll content could be attributed to a stomatal limitation (Flanangan and Jeffries, 1989). However, the stomatal limitations imposed by gradual

salt stress could result in an increase in water use efficiency, thus, affording an ecological stomatal advantage to plants under arid environment. However, an immediate effect on stomatal closure is the limitation of carbon flux to the mesophyll which reduces chlorophyll content (Campbell, 1990). The stomatal aperture of a given plant species matches the photosynthetic capacity in order to optimise the balance between water loss and carbon uptake (Lloyd *et al.,* 1990). Generally, stomatal closure due to any factor reduces the CO_2:O_2 ratio in the mesophyll cells and, thus, directly inhibits CO_2 fixation (Lechno *et al.*, 1997).

Various studies have demonstrated that chlorophyll content in pepper cultivars is also sensitive to various salinity levels (De Pascale *et al*., 2000; Lee, 2006). Salinity effects on chlorophyll content vary substantially between plant species. Chlorophyll content depends on leaf chlorophyll content, stomatal conductance and is linearly correlated with the N-content of leaves (Dingkuhn *et al.,* 1992). Salinity inhibits chlorophyll content activity, possibly by reducing the activity of ribulose-1, 5-bisphosphate (Bongi and Loreto, 1989), which is important in the Calvin Cycle (Campbell, 1990).

2.3.3 Damage threshold levels of salinity ions

The damage threshold levels of salinity ions can be defined as the maximum EC_e, where crop yield begins to decline for subsequent each unit increase in salinity (Grattan and Mass, 1985; Mass and Hoffman, 1977). The damage threshold is a function of plant species and age. De Pascale *et al*. (2000) demonstrated that for *C. annum* cultivar Laser, salinity damage threshold for yield reduction was 1.4 dS/m. This observation supported

that of Mass and Hoffman (1977), who developed salinity damage threshold models for various crops. The damage threshold for citrus was also 1.4 dS/m (Maas, 1993), whereas the two estimates for salt-sensitive and salt-tolerant pepper (*C. frutescens*) cultivars were 1.5 dS/m and 1.8 dS/m, respectively (Chartzoulakis and Klapaki, 2000). An EC_w-value of less than 7.63 dS/m was demonstrated to be critical for almost all growth characters observed in the two pepper cultivars, with the highest reductions of approximately 50% (Van der Beek and Ltifi, 1991). However, fruit and shoot mass, along with root: shoot ratios were not significantly affected by 7.63 dS/m in the two pepper cultivars studied (Van der Beek and Ltifi, 1991).

Certain crops have a wide range of salinity damage threshold levels. For instance, the salinity damage threshold level of strawberries ranges from 0.26 to 0.89 dS/m (Awang and Atherton, 1995). Each unit of salinity above the maximum range resulted in 19% and 31% decrease in the number of crowns and inflorescences, respectively, with subsequent fruit yield reduction of 18% (Awang and Atherton, 1995).

Generally, the height of the plant appears to have an insignificant role in responses of plant growth to salinity. Jamum (*Syzygium cumini*), which is the tallest tree indigenous to India, had significant decreases in stem diameter, plant height and number of leaves at 16-20 dS/m, resulting into overall 66% reduced dry matter yield (Patil and Patil, 1983).

2.3.4 Soil osmotic potential

The primary effect of salinity is to restrict the availability of soil water to plants through alteration of osmotic potentials in the soil (Bohn *et al.*, 1985). Plants absorb water and dissolved nutrients from the soil through root hairs, along a decreasing osmotic gradient, which moves from a lower concentration of ions to a higher concentration of ions. Increasing soluble salt content in soil lowers the osmotic potential, resulting in less water flowing from the soil into plants, thus reducing the amount of total available moisture in the effective root-zone (Donahue *et al.*, 1990). Water-stress induced by salinity may influence plant growth through the adverse effect on dry matter partitioning, cell expansion, cell division, leaf chlorophyll content and transpiration (Greenways and Munns, 1980; Mass and Hoffman, 1977).

2.3.5 Salinity effect at cellular level

When salt-sensitive plants are exposed to high levels of salt, particularly under conditions of poor aeration, energy-dependent processes that normally exclude Na and Cl ions from xylem fail and it is likely that these ions are transported to shoots (Drew and Dikumvin, 1985). The presence of salt in water increases energy needed to remove water from the soil into plant roots (Killham, 1994).

Superimposed upon the matrix potential stress in soil is the osmotic stress due to the presence of dissolved salts in soil solutions, where a 0.1 M solution of KCl produces a water potential of approximately 0.1 MPa (Killham, 1994). In addition to cellular exclusion mechanisms, plants have other mechanisms for avoiding salinity effects, for

instance, leaf abscission, which is an avoidance mechanism that assists plants to get rid of excess salt ions (Brouwer and Dewitt, 1969; Quist and Williams, 1999). In most cases, plants respond to soil water stress through adjustment of cell turgor pressure and cell osmotic potential (Killham, 1994; Mashela and Nthangeni, 2002). To minimise damage of cell organelles through excessive turgor pressure, cells increase the accumulation of organic solutes in exchange of releasing osmoticum ions from vacuoles (Killham, 1994; Mashela and Nthangeni, 2002; Plaut *et al.*, 2000). The osmotic adjustment serves as a means of maintaining turgor pressure under water stress and is termed dehydration avoidance (Killham, 1994).

Using a special split-root pruning technique, Mashela and Nthangeni (2002) demonstrated that the concentration of osmoticum ions (K, Na, Cl) in citrus roots was inversely proportional to the concentration of organic solutes, which was in agreement with the concept of dehydration avoidance. The accumulation of sucrose in root cells, with the resultant displacement of osmoticum ions, was used to describe a mechanism through which *T. semipenetrans* infection reduces all osmoticum ions in citrus roots and K in leaves, but increased Na and Cl in leaves (Mashela and Nthangeni, 2002). Generally, under salinity pepper roots contained more Na than Cl ions, whereas the opposite was true in leaves (Chartzoulakis and Klapaki, 2000).

2.3.6 Salt tolerance in crops

Generally, salt tolerance in crops is defined as the ability of roots to prevent transportation of excess Cl and/or Na ions to shoots (Castle *et al.*, 1989; Cooper, 1961).

The first report on salt tolerance in Citrus species was in Marsh grape fruit grafted on *Severinia buxifolia* in Riverside, California (Webber, 1948). However, *S. buxifolia* was not subsequently used as a salt-tolerant rootstock due to its sensitivity to Trestiza virus disease (Cooper, 1961). In *Capsicum* species salt tolerance has been described in terms of yield reduction, mortality of seedlings and reduction of root mass (Van der Beek and Ltifi, 1991).

The shoot appears to have little, if any, role in the exclusion of excess Cl and/ or Na ions from being transported to leaves (Behboudian *et al.,* 1986; Cooper *et al*., 1952). Roots are the major regulator in the exclusion of excess salinity ions being transported from roots to shoots (Behboudian *et al*., 1986). The mechanism involved in the exclusion of salinity ions is currently believed to be linked to low sucrose in roots, where the higher sucrose concentrations eliminate this physiological response (Mashela and Nthangeni, 2002), in agreement with dehydration avoidance mechanism (Killham, 1994).

Under conditions where sucrose is not diverted to roots due to increased root/shoot ratios, like when roots are infected with the root-knot nematodes, exclusion of salinity ions in roots is believed to be from the xylem in stems into the phloem, where the ions are translocated to roots (Kramer *et al.,* 1977; Läuchli *et al.,* 1974; Läuchli and Wieneke, 1978). Also, under low concentration of Na in shoots, the ion may be translocated from shoots to roots (Greenway and Munns, 1980). Genetic engineering to produce high concentrations of intercellular solutes that are compatible with the enzyme machinery of plant cells has been viewed as one of the most promising ways of producing plants that

might be suitable for arid and saline soils (Socials *et al.*, 1997). However, there is currently no evidence of successes regarding this technology.

Vascular plants can withstand high salinity through either salt exclusion or salt inclusion (Flowers *et al.*, 1977). Salt excluders possess the ability to exclude salt from certain organs, so that membrane selectivity favours the uptake of K over Na ions (Baalbaki *et al*., 2000). Salt accumulators are able to cope with the uptake of high salt concentrations by tolerating high levels of intracellular salts, which is a common characteristic for halophytes (salt-loving plants) that have salt-resistant cell membranes (Baalbaki *et al*., 2000; Green and Munns, 1980). Another common tactic used by plants in salt-tolerance is the removal of excess salt entering the plant, where plants can take up ions but avoid their injurious effects by compartmentalization, extrusion and/or increased succulence. Tomato plants, for instance, are highly tolerant to high levels of Na ions because these ions are compartmentalized in fruit (Johansen, 1987). In pepper, Na ions, even at high salt levels, are compartmentalised in roots (Chartzoulakis and Klapaki, 2000). Due to the ability to compartmentalise salinity ions in roots, certain *Capsicum* species can tolerate up to 100 mM NaCl in nutrient solutions (Chartzoulakis and Klapaki, 2000). However, lack of this ability in most Citrus species, renders this genus highly sensitive to salinity ions, which accumulate in leaves (Mashela *et al*., 1992b).

The ability to accumulate salinity ions in roots can be eliminated by decreasing the root:shoot ratio. Mashela and Nthangeni (2002) observed that when the root:shoot ratios decreased, more sucrose molecules were translocated to roots, resulting in the

displacement of K, Na and Cl ions, as a measure to prevent osmotic damages to cells. During the process, Na and Cl ions were transported to leaves, where their accumulation reached phytotoxic concentrations. Generally, the decrease in root:shoot ratios occur as a consequence of infection by certain plant-parasitic nematodes, mychorrhiza and various root pathogens (Graham and Syvertsen, 1989; Mashela and Nthangeni, 2002).

2.3.7 Essential roles of salinity ions

Plants require 16 essential nutrient elements, which are described as elements without which a plant cannot successfully complete its normal life cycle (Epstein, 1972). The two salinity ions, which serve as essential nutrient elements, are required in small quantities. The 2 ppm Cl in the soil, required by all vascular plants as an essential plant nutrient element (James *et al*., 1970), plays a role in the evolution of O_2 in Photosystem II during the light phase of chlorophyll content (Bove *et al*., 1963). Photosystem II is a photon reaction centre (P680 nm) where solar energy is absorbed, with the subsequent breaking of water molecules so that the excited electrons do not have to return to P680 reaction centre, but are transported through a series of acceptors to P700 reaction centre (Campbell, 1990; Nilson and Orcutt, 1996).

Chloride ion deficiency rarely occurs in plants because the high concentrations of chlorine ions present in the atmosphere as gases (Cl_2) are eventually washed down by rain, with the result that Cl ions are sufficiently high to meet the 4-10 kg/ha/year required by vascular plants (Reisenauer *et al*., 1973). Generally, under greenhouse conditions, toxicity symptoms of Cl ion deficiency are chlorosis of young leaves, which normally

occur initially at the extreme leaf tip and overall wilting of plants (Broyer *et al*., 1954; Johnson *et al*., 1957). The critical Cl ion deficiency range in plants is 70-100 ppm Cl dry tissue basis (Bohn *et al*., 1985).

Sodium ion is an essential nutrient for some C4 plant species (Brownell and Crossland, 1972). Sodium ion increases the activity of phosphoenolpyruvate (PEP) carboxylase, which is the primary carboxylating enzyme in C4 chlorophyll content (Shomer-Ilan and Waisel, 1973). Along with K, the major non-essential role of both Na and Cl is in regulating osmotic potential of cells (Mengel and Kirkby, 1978; Waisel, 1972). The three ions are collectively called osmotically-active ions or simply osmoticum ions (Waisel, 1972). The osmotically-active ions may affect plant growth through their influence on osmotic potential of cells. For instance, decreasing osmotic potential in the soil reduces plant growth. Osmotic potential threshold levels where plant growth ceases have been characterized for certain plant species (Boyer, 1970). Generally, growth stops when cellular water potential is below −0.65 MPa in the soil (Hsaio, 1973).

2.3.8 Benefits of salinity on fruits

The feasibility of using non-saline water to irrigate moderately salt sensitive crops or to establish more tolerant crops during their sensitive growth stage and using more saline water after plants have reached more salt-tolerant stages of growth has been tested in arid and semiarid regions (Rhoads, 1989). Saline water applied during reproductive stage in certain crops increases sugar content and soluble solids in the produce (Pasternak *et al*., 1986). Processing tomato in Israel, for instance, has been grown with non-saline and

saline water during vegetative and fruiting stages, respectively. This tactic is associated with increased fruit quality because of higher soluble solids in fruits (Grattan *et al*., 1987). Applying saline water to safflower (*Carthamus tinctorius*) at maturity increased the proportion of favourable fatty acids (Irving *et al* 1988). Also, soluble solids of spears in asparagus (*Asparagus officinalis*) were increased from 95 to 108 mg/ℓ when soil salinity approached 21 dS/m (Francois, 1987). Although Na in *Capsicum* species is also compartmentalised in shoots (Chartzoulakis and Klapaki, 2000), there is currently no record on the effect of salinity on the quality of fruits in pepper.

2.4 Plant-parasitic nematode in pepper production

Worldwide, the root-knot nematodes (*Meloidogyne* species) are economically important pests to most crops (Ferraz and Brown, 2002). Generally, the root-knot nematodes complete their life cycles in 19 days when soil temperature lies between 25°C and 28°C (Sikora and Fernandez, 2005). By 2006, 92 nominal *Meloidogyne* species had been described, of which 47 had been described during the past two decades (De Waele and Elsen, 2007). Due to the advances in molecular diagnostic techniques, the remaining 45 species were described during this decade.

The use of resistant-plant cultivars is considered one of the most effective and environmentally safe alternatives to synthetic chemical methods. In pepper (*Capsicum annuum*), studies on host-status to *Meloidogyne* species had shown that, in general terms, pepper was parasitized by all *M. incognita* races but was resistant to *M. javanica* (Hartman and Sasser, 1985). However, some authors have reported that *M. javanica*

parasitized both *C. annuum* and *C. frutescens* (Ahmad *et al*., 1998; Rammah and Hirschmann, 1990; Stephan, 1988).

The first resistance gene identified in pepper, named the *N* gene, conferred dominant resistance *to M. incognita*, *M. javanica* and *M. arenaria* (Hare, 1956; 1957). The *N* gene resistance was thermostable, which implies that it was effective even at temperatures as high as 32°C (Thies and Fery, 2002). Hendy *et al*. (1985) demonstrated that at least five different genes were responsible for resistance in *C. annuum* to *M. incognita, M. javanica* and *M. arenaria,* with the main genes involved being *Me1, Me2* and *Me3*. Pochard *et al*. (1986) argued that gene *Me5* was the main gene involved in the resistance of pepper to *M. incognita,* having two complementary genes *Me3* and *Me4*. However, Castagnone-Sereno *et al*. (2001) observed that *Me1* was more effective than *Me3* in conferring resistance to *M. arenaria, M. incognita* and *M. javanica.* Fery and Dukes (1996) demonstrated that in some cases resistance in *C. annuum* to *M. incognita* was conferred by a dominant gene, whereas in other cases it depended on two genes, one dominant and the other recessive. Generally, the latter confer a higher level of resistance.

Various pepper cultivars that are resistant to *Meloidogyne* species are commercially available. However, loss of their effectiveness forces a continuous search for new resistance genes. The durability of resistance generally depends on its agronomic management and on the virulence of the nematode populations in different ecosystems (Blok *et al*., 1997; Castagnone-Sereno, 2002a, 2002b; Kaloshian *et al*., 1996; Tzortzakakis *et al*., 1999; Verdejo-Lucas *et al*., 1997). In respect to nematode virulence,

the DNA polymorphisms linked to virulence that would allow the development of molecular markers to differentiate virulent and avirulent strains has been difficult to identify in nematodes. Therefore, nematode virulence has been slow to characterise by molecular methods (Semblat and Castagnone-Sereno, 2001).

Recently, Bello *et al*. (2004) modified the Differential Host Test of North Carolina (Hartman and Sasser, 1985) and developed the bioassay that allows the characterisation of *Meloidogyne* populations virulent to resistant cultivars of pepper and tomato. In most pepper studies, the *M. incognita* isolates had not been reported to the nematode race level. Nematode races are morphologically identical and can only be separated using host differential and molecular markers tests (Bello *et al*., 2004; De Waele and Elsen, 2007; Hartman and Sasser, 1985).

2.5 Salinity-pest interactions

Pests such as fungi and nematodes are known to interact with salinity to reduce citrus yield (Graham, 1990; Mashela *et al*., 1992a). Similarly, salt-mycorrhiza interactions have been established in citrus (Graham and Syvertsen, 1989). In this review, only salt-nematode interactions are reviewed.

2.5.1 Effect of salinity on nematode densities

Generally, regions with salinity problems have high nematode numbers (Cohn, 1976). Classical trials on nematode-salt interactions under field conditions on various crops showed that nematode numbers were high in salt-treated plots (Machmer, 1958). Mashela *et al*. (1992a) conducted a series of *in vitro* trials using salt solutions to verify if indeed

salt increased nematode egg-hatch. In the trials, salt reduced nematode egg-hatch and mobility. However, when salt stress was removed through rinsing in tap-water, nematode egg-hatch and mobility increased.

Generally, under field conditions salt accumulates during non-rainy season when plants are irrigated with poor quality water and during this period, nematode numbers are generally low. However, during rainy seasons, salt is leached out of the rhizosphere, resulting in improved root growth, with subsequent high nematode numbers. Consequent greenhouse trials confirmed that cyclic salinity increased population densities of *T. semipenetrans*, whereas continuous salinity reduced numbers of this nematode (Mashela *et al*., 1992a).

2.5.2 Responses of nematode resistance to cyclic salinity

Most salinity-nematode resistance studies were conducted in citrus since this crop is highly sensitive to both nematodes and salinity. All commercially available *T. semipenetrans*-resistant citrus rootstocks are sensitive to salinity, whereas all salt-tolerant citrus rootstocks are sensitive to *T. semipenetrans* (Castle *et al.*, 1989; Mashela and Nthangeni, 2002). Earlier, Mashela *et al*. (1992b) demonstrated that highly nematode-resistant citrus rootstocks like *Poncirus trifoliate* and its hybrids such as Swingle citrumelo (*Citrus paradisi x P. trifoliate*), Carrizo citrange (*C. sinesis* x *P. trifoliate*) and Troyer citrange (*P. trifoliate* x *C. sinesis)* which are highly sensitive to salinity, lose their ability to suppress the reproduction of *T. semipenetrans* when exposed to cyclic salinity.

Results of the trial also indicated that salinity reduced the fecundity of the citrus nematode.

Resistance to nematodes is described in terms of the reduced reproduction when plants are inoculated with nematode isolates (Seinhorst, 1966). Different mechanisms of resistance to nematodes have been explained as either being due to nematode-infection induced chemicals or preformed chemicals. The most studied mechanisms of resistance are induced by invaders. One such mechanism, hypersensitivity, is a condition where a few cells around the invader die so that the invader can no longer feed or move to other feeding sites within the root system (Cruickshank, 1980).

2.5.3 Nematode infection reduces salt tolerance

Various studies demonstrated that in regions with salinity problems, which also have high nematode numbers, citrus leaves had Na content above the 0.10% physiological damage threshold level, whereas the Cl content was above the 0.25% threshold level (Cooper, 1961; Cooper *et al*., 1952; Mashela, 1992). However, most greenhouse studies with the citrus nematode could not confirm the field results on nematode-salinity interactions in citrus (O'Bannon and Esser, 1985; Van Gundy and Kirkpatrick, 1964). In all field studies, samples showed that in areas with slow decline of citrus the citrus nematode numbers were quite high, with more than 4 000 juveniles per g root (Cohn, 1976). Under greenhouse conditions, inoculation was with less than 500 juveniles per pot (Van Gundy and Kirkpatrick, 1964). Mashela (1992) argued that the short-term experiments that Van Gundy and Kirkpatrick (1964) conducted in the greenhouse had low nematode numbers

and could, therefore, not be expected to induce any noticeable effect on plants, more especially since *T. semipenetrans* was a non-aggressive nematode (O'Bannon and Esser, 1985). Subsequent short-term linear trials showed that for effects of *T. semipenetrans* to be noticeable in citrus seedlings one should inoculate with 60 000 to 90 000 juveniles and eggs per plant (Mashela, 1992).

Infection with high nematode numbers significantly increased leaf Na and Cl contents to ionic levels far above the threshold for visible leaf burn (Mashela *et al*., 1992b). Also, infection of salt-tolerant citrus rootstocks using high numbers of *T. semipenetrans* resulted in the elimination of the ability of the roots to exclude the transportation of the Na and Cl ions to leaves (Mashela *et al*., 1992b). On the other hand, subjecting citrus to cyclic salinity eliminates resistance to *T. semipenetrans* in rootstocks that are resistant to this nematode. An interesting feature is that all citrus rootstocks that are resistant to *T. semipenetrans* are not tolerant to salinity, and *vice versa*.

Sanogo (2004) noted that in salinity-*Phytophthora* interaction on pepper production, salinity reduced sporangia and zoospore formation of *P. capsici* from 85% and 93%, respectively. The results suggested that salinity might predispose susceptible pepper cultivars to infection by *P. capsici.*

2.5.4 Effect of nematodes on osmoticum ions

Potassium, Cl and Na ions are collectively called osmoticum ions (Mashela, 2007). The three ions are used by plant cells to regulate intracellular osmotic potential (Mashela,

2007). Nematode-infected citrus trees contained significantly lower concentrations of K in both leaf and root tissues and lower Na and Cl ions in roots, but increased Na and Cl ions in leaves (Mashela, 1992; Mashela and Nthangeni, 2002; Mashela *et al*., 1992a, 1992b). Mashela (1992) linked the imbalances of osmoticum ions in citrus-infected with *T. semipenetrans* with slow decline of citrus, which is a disease induced by *T. semipenetrans* (O'Bannon and Esser, 1985). Fouche *et al*. (1977) observed that citrus trees heavily infected with *T. semipenetrans* did not respond to fertilisation. Mashela and Nthangeni (2002), using split-root and girdling, provided the first plausible explanation on how *T. semipenetrans* infection results in the imbalances of osmoticum ions.

2.5.5 Mechanism of nematode alteration of osmoticum ions

O'Bannon and Esser (1985) argued that the accumulation of Na and Cl ions in leaves of nematode-infected citrus was exclusively due to physical damage to the cortex and endodermis during nematode feeding, with the damage resulting into the loss of semi-permeability of cell membranes. Although the citrus nematode does not kill the trees, when the nematode eventually dies, under high nematode-infection, the root cortex of the infected region "sloughs-off", resulting in the wilting of the affected rootlet. O'Bannon and Esser (1985) proposed that due to "sloughing-off" after the death of nematode females on roots, Na and Cl ions were sucked into the plant through wounds, with non-structural carbohydrates being leached into soil solutions. Mashela and Nthangeni (2002) argued that the behavioural pattern of osmoticum ions in citrus infected by nematodes could not be explained in physical but in physiological terms.

Mashela and Nthangeni (2002) proposed that the behavioural pattern of reduced K in both leaves and roots suggested that this ion was transported from cells into the soil in nematode-infected plants. However, the accumulation of Na and Cl in leaves with the reduced concentrations of the two ions in roots suggested that the two ions were displaced from roots, but could not exit the root cells. In other words, the two ions could only be channelled to leaves, where they eventually accumulated to toxic levels, with the plant getting rid of the toxic ions through leaf abscission. The latter had been referred to as an avoidance mechanism, which is a form of plant defence against harmful ions at intolerable levels.

Using a split-root-pruning technique, Mashela and Mphosi (2002) noted that it took approximately 14 days for the citrus seedlings to re-establish the normal root: shoot ratios after severing half of the root system. In this study, the major argument was that any measurement that could be taken after the re-establishment of the root: shoot ratio, would no longer be measuring the effects of the reduced root: shoot ratio on the physiological parameter such as chlorophyll content. In other responses like accumulation of ions or carbohydrates, there may be a time-lag after pruning, whereas in others such as transpiration and chlorophyll content, the response to pruning half of the root system may be immediate and measurable until the root: shoot ratio was re-established.

The four treatments, used in the testing of the mechanism involved in the accumulation of ions were untreated control, severing of half the root system, nematode and stem girdling, with each treatment being with and without low salinity (Mashela and Nthangeni, 2002).

After allowing sufficient time for nematodes to establish, one-half of the root system was abscised and the stem of appropriate seedlings girdled to ensure that non-structural carbohydrates were not translocated to the root system. Root pruning simulated high nematode infection in order to force more non-structural carbohydrates to move to the remaining half-root system. On the other hand, stem girdling prevented the movement of non-structural carbohydrates to roots, but instead improved their accumulation in shoots.

Seven days after root-pruning, accumulation of K, Na and Cl ions in pruned and nematode-inoculated plants followed the same imbalanced patterns, although the magnitudes were higher under pruning, due to the extreme stress induced through this treatment (Mashela and Nthangeni, 2002). The growing mixtures under pruned treatments had higher K when compared with the controls. Potassium, it was argued, was released into soil solution to regulate turgor pressure inside root cells. On the other hand, accumulation of non-structural carbohydrates in girdled stems induced opposite responses in the partitioning of osmoticum ions; Na and Cl were high in roots and low in leaves, whereas K levels did not differ (Mashela and Nthangeni, 2002).

In reduced root:shoot ratios, high non-structural carbohydrate concentrations are mobilised toward the root system for root re-generation and the re-establishment of the normal root:shoot ratio. Pruning results showed that the "sloughing-off" that accompanies the death of *T. semipenetrans*, had a pruning-like effect, which reduces the root:shoot ratio. Stem girdling treatments in these trials showed that when non-structural

carbohydrates accumulated in shoots, the behaviour of osmoticum ions was different from that when carbohydrates accumulated in roots.

The trials provided a working model that explained the partitioning of osmoticum ions in nematode-infected plants. Based on the model, the mechanism whereby *T. semipenetrans* infection changed the partitioning of osmoticum ions was a four-step process (Mashela and Nthangeni, 2002):

1. Nematode infection reduces the root:shoot ratio of citrus through "sloughing-off" effect, which has a root-pruning effect.
2. The plant then diverts more organic osmolytes to roots in response to the reduced root:shoot ratios in order to regenerate the root system.
3. In roots, high concentrations of organic osmolytes decrease water potential in cells, which results in more water being mobilised into the root cells due to their lower water potential, with increasing turgor pressure.
4. As a measure to avoid the damaging turgor pressures in root cells, excess organic osmolytes are stored in non-osmotic forms such as starch, whereas inorganic osmolytes (K, Na, Cl) are displaced from vacuoles of root cells, with Na and Cl accumulating in leaves and K being transported out of root cells into soil solutions through exocytosis.

2.5.6 Epstein's equilibrium model

The model proposed by Mashela and Nthangeni (2002) substantially differed from that proposed by Epstein (1972). The Epstein (1972) model demonstrated that Na and Cl ions

were released into solutions from abscised roots until an ionic equilibrium was achieved. Obviously, the split-root-pruning technique did not confirm Epstein's model, with the exception for K. The ions observed in equilibrium studies of Epstein's model were probably mainly from the intracellular spaces of the cortical cells, where the ion concentration is always in equilibrium with those in soil solution (Salisbury and Ross, 2000).

2.6 Disease triangle theory

According to the disease triangle theory (Agrios, 1994), a disease is a function of pathogen, host and environment, with the intensity of the disease depending on the exposure time. Slow decline of citrus, which is a disease caused by *T. semipenetrans*, is more severe in regions with salinity than those without salinity problems (Duncan *et al*., 1995; Mashela, 1992).

2.7 Conclusions

The review of this literature demonstrated that *M. incognita* and salinity are limiting factors in crop production. Also, it is not known whether the *Capsicum* species used in Limpopo Province, South Africa, were resistant to *M. incognita* race 2, which is predominant in this province. Also, it was demonstrated that it is not documented whether the *Capsicum* species in use were tolerant to salinity. Finally, the interaction of *M. incognita* race 2 and salinity over-time was not documented. Limited information in the cited areas makes it difficult to include the pepper cultivars as rotation crops in areas infested with *M. incognita* race 2 with or without salinity.

CHAPTER 3
RESPONSES OF TWO *CAPSICUM* CULTIVARS TO *MELOIDOGYNE INCOGNITA* RACE 2

3.1 Introduction

Pepper (*Capsicum* species) cultivars thrive in areas with tropical climate and sandy soil, which are characterised by having high population densities of the southern root-knot nematode (*Meloidogyne incognita*). Incidentally, it is not possible to produce an economic crop yield in nematode-sensitive pepper cultivars without managing the population densities of this nematode (Thomas *et al*., 1995; Di Vito *et al*., 1985). Use of resistant cultivars since the advent of restrictions on the use of synthetic nematicides is the management strategy of choice in soil health practices. Using resistant cultivars in the management of *M. incognita* is complicated by its wide distribution, existence of wide host range, and the existence of races (Hartman and Sasser, 1985). Nematode races are morphologically similar and are traditionally separated using differential host tests.

Four races of the southern root-knot nematode, namely, *M. incognita* races 1, 2, 3 and 4 are widely distributed in tropical areas with sandy soils (Di Vito *et al*., 1985; Hartman and Sasser, 1985; Thomas *et al*., 1995). *Meloidogyne incognita* race 3 is widely distributed throughout the pepper-producing areas in the south-western United States (Thomas *et al*., 1995), whereas *M. incognita* races 3 and 4 are most prevalent in South America and *M. incognita* race 1 is dominant in Spain (Buena *et al*., 2006). In Limpopo Province, South Africa, tropical areas with sandy soil have predominantly *M. incognita* race 2 (Mashela, 2002).

Most nematode-resistant pepper cultivars, developed in South America and California, are resistant to the races that occur in those parts of the world. The existence of intercontinental differences in *M. incognita* races increases the risk of using an *M. incognita*-resistant cultivar for a nematode race that the cultivar was not developed for. For instance, the misidentification of the citrus nematode (*Tylenchulus semipenetrans*) in South Africa as Mediterranean race (Cohn, 1976), resulted in widespread use of *Poncirus trifoliata* rootstock. In actual fact, the *P. trifoliata* rootstock is not resistant to the South African *T. semipenetrans* race, which had been empirically shown to be Poncirus race (Kwaye *et al*., 2008).

Capsicum annuum cv. Serrano and *C. frutescens* cv. Capistrano were initially released as nematode-resistant pepper cultivars in South America (Robertson *et al*., 2006). Serrano and Capistrano are commercially widely used in crop rotation systems as nematode-resistant cultivars in Limpopo Province (Martindale Seedlings CC: personal communication). However, the host-status and host-sensitivity of the two pepper cultivars to *M. incognita* race 2 are not documented. The objective of this study was to determine the host-status and sensitivity of Serrano and Capistrano to *M. incognita* race 2, thus, establishing their suitability for use as nematode-resistant cultivars in crop rotation systems.

3.2 Materials and methods

Study site: The study was conducted under microplot conditions at the Horticultural Unit of the University of Limpopo (23^{o}53”10’S, 29^{o}44”15’E). Plots comprised 30-cm-

diameter plastic pots, inserted into 28-cm-deep holes at 1.0 m x 1.0 m inter-row and intra-row spacing (8 333 plants/ha), with border rows. Pots were filled with 10-ℓ steam-pasteurised Hutton soil collected from the topsoil of the dug holes, comprising 65% sand, 30% clay, 5% silt; 1.6% organic C, EC_e 0.148 dS/m and pH 6.5.

Preparation of plant materials: Three-week-old pepper seedlings of Serrano and Capistrano were purchased from Martindale Seedlings CC (Tzaneen, RSA). Since the two cultivars are classified under different species, each was used in a separate experiment, but conducted concurrently and repeated three times. Experiment 1, Experiment 2 and Experiment 3 were each conducted in autumn 2007, spring 2007 and summer 2007/2008, respectively.

Preparation of inoculums: Isolates of *M. incognita* race 2, obtained from the Agricultural Research Council (ARC): Small Grain Institute – Potchefstroom, were cultured on tomato (*Lycopersicon esculentum*) cv. Floradade in a greenhouse. Nematode eggs and juveniles when required were extracted from tomato roots in 1% NaOCl and incubated for 5 days using the modified Baermann trays to obtain second-stage juveniles (J2s) for inoculation (Hussey and Barker, 1973; Rodríguez-Kábana and Pope, 1981).

Experimental design: The two nematode levels (N_o, N_1) were arranged in a randomised complete block design, with 10 replications in Experiment 1 and then with 8 replications in Experiment 2 and Experiment 3. Blocking was done for slope and shading from trees late in the afternoon. Inoculation with nematodes was done on transplanting day by

applying 11 000 J2s and eggs of *M. incognita* per pot into 10-cm-deep holes around the stem of the seedling using a 20-mℓ-plastic syringe.

Cultural practices: Weeds were controlled using hand-hoes and foliar pests, such as aphids and whiteflies, using Metasystox-R (56) when necessary. Plants were irrigated using 1-ℓ tap-water per plant every other day and fertilised using 2.5 g of 2:3:2 N:P:K (22% active) per plant 7 days after planting, which was repeated at first fruit harvest.

Data collection: Fruits were regularly collected and recorded. Before termination of the experiments, at 120 days after treatment, plant height and chlorophyll content were measured. Chlorophyll content was measured from three fully-developed leaves using a hand-held chlorophyll meter between 09h00 and 10h00 a day before harvesting the experiments. Stems were cut at the crown and diameter measured at 10-cm distal to the severed end using digital vernier callipers. Pots were emptied to remove roots, which were immersed in water to free soil particles, and excess water was removed from the roots by pressing between two pieces of roll-paper and weighed. Roots were evaluated for galls and necrotic spots, cut into pieces, put in 1% NaOCl and mechanically shaken for 60 seconds. Samples were sieved through nested sieves of 150-µm and 25-µm openings, eggs plus juveniles were collected from the 25-µm-openings sieve. Approximately 250-mℓ soil samples were collected from each pot for nematode determination using the sugar-floatation extraction method (Coolen, 1979). Nematode samples were temporarily stored at 5^{o}C during counting under a microscope. Soil nematode numbers were converted from 250 ml soil to the total 10 000 ml soil/pot and

combined with nematodes per total root per plant for the determination of final population of nematodes (Pf). Shoots were oven-dried at 70^{o}C for 72 hours for determination of dry shoot matter. Nematode numbers were expressed per total roots per plant and per total soil volume per plant. Soil pH and electrical conductivity (EC) were measured using a pH meter and an EC meter (Rhue and Kidder, 1983).

Data analysis: Host-status was determined using reproductive factors (RFs), which entail dividing the Pf by the initial population of nematodes (Pi). Host-sensitivity was determined by comparing various productivity parameters in treatments with and without nematodes within each cultivar and subjected to student t-test using SAS software (SAS Institute, Inc., Cary, NC 1998).

3.3 Results

Host-status results on Serrano in Experiment 1 demonstrated that the RF was less than one, whereas in Experiment 2 and Experiment 3, the RFs were all greater than unity (Table 3.1). In all experiments, the RFs of *M. incognita* race 2 on Capistrano were less than unity. Formation of root galls had completely failed in Capistrano, whereas in Serrano the developing root galls were visible at the time of harvesting Experiment 2 and Experiment 3 (data not shown).

Inoculation with *M. incognita* race 2 had no effect on fresh fruit yield of Serrano cultivar in Experiment 1 and Experiment 2, but significantly ($P \leq 0.05$) reduced this variable by 37.4% in Experiment 3 (Table 3.2). In all experiments, infection with *M. incognita* race 2

had no significant effect on fresh fruit yield of Capistrano cultivar. Relative to untreated control, infection with *M. incognita* race 2 had no effect of dry shoot mass of Serrano in Experiment 1 and Experiment 2, but significantly reduced ($P \leq 0.05$) this variable by 45.5% in Experiment 3. As in the case of fruit yield, nematode infection had no significant effect on dry shoot mass of Capistrano cultivar in all three experiments.

Table 3.1 Initial nematode numbers (P_i), final nematode numbers (P_f) and reproductive factors (RF) of *Meloidogyne incognita* race 2 on *Capsicum annuum* cv. Serrano and *C. frutescens* cv. Capistrano in micro-plots

Variable measured	Serrano			Capistrano		
	Exp 1	Exp 2	Exp 3	Exp 1	Exp 2	Exp 3
Final nematodes (P_f)	4 696	21 232	26 884	3 658	5 904	6 011
Initial nematodes (P_i)	11 000	11 000	11 000	11 000	11 000	11 000
Reproductive factor	0.43	1.93	2.44	0.33	0.54	0.55

Reproductive factor = P_f/P_i.

Compared to untreated control, infection with *M. incognita* race 2 significantly ($P \leq 0.05$) increased fresh root mass of Serrano cultivar by 35.7% and 55.7% in Experiment 2 and Experiment 3, respectively (Table 3.3). However, there was no significant effect of the nematode on fresh root mass of Capistrano cultivar in all three experiments. Relative to untreated control, infection with *M. incognita* race 2 had no effect on plant height of Serrano in Experiment 1 and Experiment 2, but significantly ($P \leq 0.05$) reduced the variable by 13.8% in Experiment 3. Nematode infection had no significant effect on plant height of Capistrano cultivar in all three experiments.

Relative to untreated control, infection with *M. incognita* race 2 had no effect on the stem diameter of Serrano cultivar in Experiment 1 and Experiment 2, but significantly ($P \leq$

0.05) reduced the variable by 16.6% in Experiment 3 (Table 3.4), but there was no significant effect of nematode in Experiment 1 and Experiment 2.

In all three experiments, nematode infection had no effect on stem diameter of Capistrano cultivar. Infection with *M. incognita* had no effect on chlorophyll content of Serrano in Experiment 1, but significantly ($P \leq 0.05$) reduced chlorophyll content of this cultivar by 11.7% and 15.5% in Experiment 2 and Experiment 3, respectively. In all experiments, nematode infection had no effect on this variable on Capistrano cultivar.

Relative to untreated control, nematode infection had no effect on soil EC in all Serrano experiments, whereas there was a significant ($P \leq 0.05$) increase of this variable by 18.2% in Experiment 1 under Capistrano cultivation (data not shown). Similarly, *M. incognita* race 2 had no effect on soil pH in all experiments, for both Serrano and Capistrano cultivars (data not shown).

3.4 Discussion

The RF provides a good estimate of nematode reproduction potential, and therefore an indication of the host-status of a plant to the nematode being studied (Seinhorst, 1967). Plant-parasitic nematodes can reproduce only when the host plant is suitable for feeding. Generally, the RFs above unity indicate that the host allows a particular nematode to reproduce, whereas the ones below indicate that the host is resistant (Seinhorst, 1967). In Serrano, there was 67% evidence that the cultivar allows *M. incognita* race 2 to reproduce

and develop, and therefore, for all purposes and intensions of this study, Serrano is a host to *M. incognita* race 2. In contrast, Capistrano is a non-host to *M. incognita* race 2.

Table 3.2 Fresh fruit yield and dry shoot mass of *Capsicum annuum* cv. Serrano and *C. frutescens* cv. Capistrano in micro-plots with and without *Meloidogyne incognita* race 2

Treatment	Fresh fruit yield (g)							Dry shoot mass (g)					
	Serrano			Capistrano				Serrano			Capistrano		
	Exp 1	Exp 2	Exp 3	Exp 1	Exp 2	Exp 3		Exp 1	Exp 2	Exp 3	Exp 1	Exp 2	Exp 3
Control	195.02	76.63	248.81	159.03	165.26	258.68		19.47	23.81	43.64	10.30	18.71	27.37
Nematode	207.63	92.54	155.79	145.43	145.99	256.96		20.32	21.78	23.79	9.53	19.44	25.99
Relative effect (%)[y]	6.47	20.76	−37.39	−8.55	−11.66	−0.66		4.37	−8.52	−45.49	−7.48	3.90	−5.04
Std error	15.41	29.29	38.33	24.84	40.04	44.87		1.72	2.99	3.80	1.67	3.60	4.68
t-value[z]	2.03 ns	2.05 ns	2.05**	2.03ns	2.05ns	2.05ns		2.03 ns	2.05 ns	2.05**	2.03ns	2.05ns	2.05 ns
N	10	8	8	10	8	8		10	8	8	10	8	8

[y]Relative effect = [(Treatment/Control) – 1] × 100.

[z] = ** and ns denote significant differences between the treatments at $P \leq 0.05$ and not significant at $P \leq 0.10$, respectively.

Table 3.3 Fresh root mass and plant height of *Capsicum annuum* cv. Serrano and *C. frutescens* cv. Capistrano in micro-plots with and without *Meloidogyne incognita* race 2

Treatment	Fresh root mass (g)						Plant height (cm)					
	Serrano			Capistrano			Serrano			Capistrano		
	Exp 1	Exp 2	Exp 3	Exp 1	Exp 2	Exp 3	Exp 1	Exp 2	Exp 3	Exp 1	Exp 2	Exp 3
Control	30.38	33.75	39.25	23.53	42.92	43.94	43.63	50.47	58.03	32.11	31.48	38.50
Nematode	29.67	45.81	61.12	23.25	39.77	55.88	45.11	50.32	50.03	31.45	27.98	36.73
Relative effect (%)[y]	−2.34	35.73	55.72	−1.19	−7.34	27.17	3.39	−0.30	−13.79	−2.06	−11.12	−4.60
Std error	2.68	3.65	6.03	3.25	5.63	8.90	2.24	2.19	2.31	1.79	1.80	3.54
t-value[z]	2.03 [ns]	2.05 [**]	2.05 [**]	2.03 [ns]	2.05 [ns]	2.05 [ns]	2.03 [ns]	2.05 [ns]	2.05 [**]	2.03 [ns]	2.05 [ns]	2.05 [ns]
N	10	8	8	10	8	8	10	8	8	10	8	8

[y]Relative effect = [(Treatment/Control) – 1] × 100.

[z] = ** and ns denote significant differences between the treatments at $P \leq 0.05$ and not significant at $P \leq 0.10$, respectively.

Table 3.4 Stem diameter and chlorophyll content of *Capsicum annuum* cv. Serrano and *C. frutescens* cv. Capistrano in micro-plots with and without *Meloidogyne incognita* race 2

Treatment	Stem diameter (mm)						Chlorophyll content					
	Serrano			Capistrano			Serrano			Capistrano		
	Exp 1	Exp 2	Exp 3	Exp 1	Exp 2	Exp 3	Exp 1	Exp 2	Exp 3	Exp 1	Exp 2	Exp 3
Control	13.37	10.74	14.13	11.06	11.63	12.60	63.16	71.97	62.50	52.13	71.06	61.42
Nematode	13.35	10.97	11.79	10.70	11.08	13.21	67.41	63.54	52.84	52.02	75.06	60.29
Relative effect (%)[y]	−0.15	2.14	−16.56	−3.25	−4.73	4.84	6.73	−11.7	−15.46	−0.21	5.62	−1.84
Std error	0.59	0.60	0.76	0.81	0.72	0.83	3.26	2.83	3.49	2.23	3.21	1.56
t-value[z]	2.03 [ns]	2.05[ns]	2.05[**]	2.03[ns]	2.05[ns]	2.05[ns]	2.03[ns]	2.05[**]	2.05[**]	2.03[ns]	2.05[ns]	2.05[ns]
N	10	8	8	10	8	8	10	8	8	10	8	8

[y]Relative effect = [(Treatment/Control) – 1] × 100.
[z] = ** and ns denote significant differences between the treatments at $P \leq 0.05$ and not significant at $P \leq 0.10$, respectively.

Generally, host-sensitivity of plants to nematode infection is determined in terms of reduction in plant yield of various parameters (Trudgill, 1991). When the nematode reproduces in a given plant but yield of various components is not reduced, the plant is tolerant to the nematode being studied (Seinhorst, 1967). In contrast, when yields of various components are reduced, the host is susceptible (Seinhorst, 1967). In Serrano, where the RFs were greater than one, the damage potential was inconsistent since both fresh fruit yield and dry shoot mass decreased in one experiment only, whereas in the other there were no effects. Failure of *M. incognita* race 2 to reduce fresh fruit yield and dry shoot mass in all experiments of Capistrano was consistent with the observed RF values, which were less than one.

In Serrano where the RFs were greater than one, fresh root mass increased, which suggested that the formation and development of nematode-induced root galls occurred. Generally, roots infected with *Meloidogyne* species in both susceptible and tolerant hosts due to the induced root-galls have much larger root mass than the uninfected roots (Dropkin, 1980; Hartman and Sasser, 1985). The larger root mass in Serrano agreed with the observed RF values that were greater than unity. Similarly, lack of significant differences in root mass of nematode-infected and non-infected roots of Capistrano were consistent with the observed RF values that were less than one.

Generally, nematode infection reduces chlorophyll content as demonstrated for *M. incognita* in henbane (*Hyoscyamus muticus)*, tomato and beans (Butool and Akhtar, 1998; Loveys and Bird 1973; Melakeberhan *et al*., 1984). The citrus nematode

(*Tylenchulus semipenetrans*) and the potato cyst nematode (*Globodera pallida*) reduced chlorophyll content in citrus and potato, respectively (Mashela and Mphosi, 1999; Schans, 1991). Decreased as a result of chlorophyll content rates in Serrano and the absence of influence on this parameter in Capistrano support our view that Serrano and Capistrano are tolerant and resistant to *M. incognita* race 2, respectively. This view recognises that in nematode tolerant cultivars, certain parameters will respond to nematode infection, whereas others will not respond. However, it may be inconceivable to relate observations in the current study to field conditions since test plants were exposed to excessively high levels of inoculum when compared to natural conditions. Seinhorst (1967) proposed that the damage potential due to nematodes in plants is a function of nematode type, nematode numbers, plant type, plant age and various environmental conditions, which estimate the factors of the disease triangle (Agrios, 1997).

Generally, infection with *M. incognita* race 2 in susceptible tomato and cowpea cultivars increased soil EC_e and reduced soil pH (Mashela, 2002; Mashela and Muedi, 2003). The reduction in soil pH was ascribed to the release of various amino acids into soil solutions, which is common in *M. incognita* (Mashela, 2002; Wallace, 1973) and *Hederodera* species (Aist and Riggs, 1969). Failure to influence the two parameters in *Capsicum* species may suggest variations in root exudates of the test plants.

The observed less than one RF values and lack of significant effect on various productivity parameters in Capistrano were consistent with the definition of nematode

resistance (Barker, 1993; Cook, 1991; Seinhorst, 1967; Trudgill, 1991). Briefly, the concept states that in resistant cultivars nematode feeding is inhibited with the result that there is no reduction in yield. In this instance, RF values were a measure of the degree of feeding since nematode growth, development and reproduction did not occur when feeding was inhibited. Generally, there are four forms of nematode resistance, namely, exuded chemicals, preformed internal physical defences, preformed internal chemical defences, and various defences triggered by nematode infection (Horsfall and Cowling, 1980). Mechanisms of resistance involved in the four different forms differ widely. In some forms, nematodes are not able to locate the roots, in others they locate the roots and start feeding, whereas in the case of *Meloidogyne* species gall formation fails (Wallace, 1973). Yet in other forms, infection is allowed, with plant cells around the nematode becoming necrotic and preventing nematode development and mobility, a resistant mechanism referred to as hypersensitivity (Kiraly, 1980). The resistant mechanism in Capistrano is not yet clear, since histopathology was not done in this study.

In conclusion, results of this study suggested that Serrano and Capistrano pepper cultivars were tolerant and resistant to *M. incognita* race 2, respectively. Therefore, Capistrano is suitable in crop rotations intended to reduce numbers of *M. incognita* race 2. In contrast, Serrano is tolerant to M. incognita race 2 and is, therefore, not suitable for use in crop rotations since it would result in the build up of nematode numbers, with high crop losses in subsequent susceptible crops.

CHAPTER 4
RESPONSES OF TWO *CAPSICUM* SPECIES TO SALINITY

4.1 Introduction

Salinity is an important growth-limiting factor for most vegetable crops (Bohn *et al*., 1985). Salinity is increasingly becoming a serious risk to contend with in crop husbandry due to increased contamination of underground water through various salts (FAO, 2005). Salt inhibits plant growth through osmotic stress, nutritional imbalance and specific ion toxicity (Alam, 1994; Cornillon and Palloix, 1997; Gunes *et al.*, 1996; Jacoby, 1994). In Limpopo Province, South Africa, salinity is increasing by becoming a limiting factor in vegetable production due to the increased reliance on poor quality borehole-water for irrigation (Mashela and Mollel, 2001). In most agricultural fields, salinity problems are cyclic in nature, accumulating in the rhizospheres during dry seasons and being leached out during rainy seasons (Mashela *et al*., 1992a).

Responses of pepper (*Capsicum* spp.) to salinity are described in terms of reduction in crop yield (Chartzoulakis and Klapaki, 2000; De Pascale *et al*., 2000; Lee, 2006; Yildirim and Güvenc, 2006). However, in cited studies the researchers did not include Ca in NaCl solutions which were used to induce salinity. Calcium ions are invariably required in salinity studies for retaining the integrity of cell membranes in roots subjected to low osmotic potentials (Epstein, 1961). Commercially, peppers in Limpopo Province include *C. annuum* cv. Capistrano and *C. frutescens* cv. Serrano, which were shown to be resistant and tolerant, respectively, to *M. incognita* race 2 (Chapter 3). However, the tolerance status of these two cultivars to NaCl + $CaCl_2$ salinity is not documented. The

objective of the study was to determine the responses of Serrano and Capistrano to NaCl + $CaCl_2$ salinity.

4.2 Materials and methods

Study site: The study was conducted under microplot conditions at the Horticultural Unit of the University of Limpopo ($23^{o}53$”10’S, $29^{o}44$”15’E). Plots comprised 30-cm-diameter plastic pots, inserted into 28-cm-deep holes at 1.0 m x 1.0 m inter-row and intra-row spacing (8 333 plants/ha). Pots were filled with 10-ℓ steam-pasteurised Hutton soil collected from the topsoil of the dug holes, comprising 65% sand, 30% clay, 5% silt; 1.6% organic C, EC_e 0.148 dS/m and pH 6.5.

Preparation of plant materials: Three-week-old pepper seedlings of Serrano and Capistrano were purchased from Martindale Seedlings CC (Tzaneen, RSA). Since the two cultivars are classified under different species, each was used in a separate experiment, which were conducted concurrently and each repeated three times. Experiment 1, Experiment 2 and Experiment 3 were conducted in autumn 2007, spring 2007 and summer 2007/2008, respectively.

Preparation of salt solutions: Approximately 0.92 mols NaCl + 0.32 mols $CaCl_2$ per m^3 were prepared for use as salt solutions.

Experimental design: The two salt levels (S_o, S_1) were arranged in a randomised complete block design, with 10 replications in Experiment 1 and 8 replications in Experiment 2 and

Experiment 3. Blocking was done for slope and shading from trees late in the afternoon. In plots with and without salt, salinity was induced through application of 1 ℓ salt solution and 1 ℓ tap-water, respectively, every other day.

Cultural practices: Foliar pests such as aphids and whiteflies were controlled using Metasystox-R (56) when necessary. Weeds were controlled using hand-hoes, and plants were irrigated to bring the readings of the Hadeco Moisture Meter from below 4 to above 7 units. Plants were fertilised using 2.5 g of 2:3:2 N:P:K (22% active) per plant 7 days after planting, which was repeated at first fruit harvest.

Data collection: Fruits were regularly collected and recorded starting from 76 days after transplanting to a day before harvest. At harvest, 120 days after initiating the salt treatment, plant height and chlorophyll content were recorded. Chlorophyll content was measured from three fully-developed leaves using a hand-held chlorophyll meter between 09h00 and 10h00 a day before harvesting the experiments. Stems were cut at the crown and diameter measured at 10-cm distal to the severed end using digital vernier callipers. Pots were emptied to remove roots, which were immersed in water to free soil particles, and excess water was removed from the roots that were separately dried with shoots at 70^{o}C for 72 hours for determination of dry root and shoot matter. Approximately 100 ml soil sample per plot was collected for soil pH and electrical conductivity (EC_e) determination. The pH meter and EC meter were used to measure the soil pH and EC_e, respectively, using the IFAS method (Rhue and Kidder, 1983).

Data analysis: Plant, soil and EC_e data were analysed using the SAS software (SAS Institute, Inc., Cary, NC, 1998).

4.3 Results

Dry shoot and root mass: Relative to untreated control, salt significantly ($P \leq 0.05$) reduced dry shoot mass of Serrano cultivar by 16.7% in Experiment 1 only (Table 4.1). The salt treatment for shoot mass did not differ from those of untreated control in all experiments of Capistrano. Relative to untreated control, salinity significantly ($P \leq 0.05$) reduced fresh root mass of Serrano cultivar by 17.6% in Experiment 1, without having an effect on root mass of Capistrano in all experiments.

Plant height, stem diameter and chlorophyll content: Relative to untreated control, salinity had no significant ($P \leq 0.05$) effect on plant height in all experiments of Serrano cultivar and Capistrano cultivar (Table 4.2). Relative to untreated control, salinity significantly ($P \leq 0.05$) increased stem diameter of Serrano cultivar by 16% in Experiment 3. However, in all other experiments, the treatment had no influence on this variable in both pepper cultivars. Relative to untreated control, in Experiment 1 salinity significantly ($P \leq 0.05$) reduced chlorophyll content in Serrano by 17.4%, without affecting this parameter in the other two experiments (data not shown). In all three experiments, salinity had no effect on chlorophyll content of Capistrano.

Soil electrical conductivity and soil pH: Salinity significantly ($P \leq 0.05$) increased soil EC in all experiments (Table 4.3). Relative to untreated control, in Serrano cultivar salinity increased soil EC by 486%, 162% and 217% in Experiment 1, Experiment 2 and

Experiment 3, respectively. Also, in Capistrano salinity increased soil EC by 656%, 152% and 300% in the three respective experiments. In Serrano, relative to untreated control, salinity increased soil pH by 5.9% in Experiment 3, but there was no effect in Experiment 1 and Experiment 2. Also, in Capistrano salinity reduced soil pH by 4.2% in Experiment 3, but had no effect in Experiment 1 and Experiment 2.

4.4 Discussion

Generally, except in one experiment of Serrano cultivar, NaCl + $CaCl_2$ salinity had no influence on fresh fruit yield of both Serrano and Capistrano. Previously, Chartzoulakis and Klapaki (2000) observed that NaCl salinity reduced fruit yield of *C. frutescens* cultivars, whereas Van der Beek and Ltifi (1991) noted that fruit yields of *C. annuum* cultivars in Tunisia were not affected by NaCl salinity. Generally, NaCl salinity alone reduced dry shoot mass of *Capsicum* species (Chartzoulakis and Klapaki, 2000; De Pascale *et al*., 2000; Lee, 2006; Yildirim and Güvenc, 2006). Also, NaCl + $CaCl_2$ salinity reduced both dry shoot and root mass in most crops (Green and Munns, 1980; Kafkafi, 1991; Mashela and Nthangeni, 2002).

In both Serrano and Capistrano cultivars, NaCl + $CaCl_2$ salinity had no influence on plant height and stem diameter in all experiments. In contrast, Chartzoulakis and Klapaki (2000) demonstrated that NaCl salinity alone significantly reduced plant height in pepper. Generally, most root stresses, which increase the translocation of solutes to roots result in reduced stem diameters in plants (Cavalcante *et al*., 2007; Mashela and Nthangeni, 2002).

Table 4.1 Dry shoot mass and fresh root mass of *Capsicum annuum* cv. Serrano and *C. frutescens* cv. Capistrano in micro-plots with and without salinity

Treatment	Dry shoot mass (g)						Fresh root mass (g)					
	Serrano			Capistrano			Serrano			Capistrano		
	Exp 1	Exp 2	Exp 3	Exp 1	Exp 2	Exp 3	Exp 1	Exp 2	Exp 3	Exp 1	Exp 2	Exp 3
Control	21.71	21.90	31.86	10.77	19.83	26.15	32.92	37.80	54.84	23.82	36.15	46.73
Salt	18.09	23.69	35.56	9.06	18.31	27.21	27.12	41.76	45.52	22.97	46.54	53.09
Relative effect (%)[y]	−16.67	8.17	11.61	−15.88	−7.67	4.05	−17.62	10.48	−16.99	−3.57	28.74	13.61
Std error	1.72	2.99	3.54	1.67	3.60	4.68	2.68	3.65	6.03	3.25	5.63	8.90
t-value[z]	2.03**	2.05 ns	2.05 ns	2.03ns	2.05ns	2.05 ns	2.03**	2.05 ns	2.05 ns	2.03ns	2.05 ns	2.05 ns
N	10	8	8	10	8	8	10	8	8	10	8	8

[y]Relative effect = [(Treatment/Control) – 1] × 100.

[z] = ** and ns denote significant differences between the treatments at $P \leq 0.05$ and not significant at $P \leq 0.10$, respectively.

Table 4.2 Plant height and stem diameter of *Capsicum annuum* cv. Serrano and *C. frutescens* cv. Capistrano in micro-plots with and without salinity

Treatment	Plant height (cm)						Stem diameter (mm)					
	Serrano			Capistrano			Serrano			Capistrano		
	Exp 1	Exp 2	Exp 3	Exp 1	Exp 2	Exp 3	Exp 1	Exp 2	Exp 3	Exp 1	Exp 2	Exp 3
Control	46.04	50.57	53.40	31.16	29.09	36.11	13.36	10.38	12.00	11.15	10.90	12.12
Salt	42.71	50.22	54.67	32.40	30.38	39.12	13.35	11.33	13.92	10.62	11.81	13.69
Relative effect (%)[y]	−7.23	−0.69	2.38	3.98	4.43	8.34	−0.07	9.15	16	−4.66	8.35	12.95
Std error	2.24	2.19	2.31	1.79	1.80	3.54	0.59	0.60	0.76	0.81	0.72	0.83
t-value[z]	2.03 [ns]	2.05 [ns]	2.05 [ns]	2.03 [ns]	2.05 [ns]	2.05 [ns]	2.03 [ns]	2.05 [ns]	2.05 [**]	2.03 [ns]	2.05 [ns]	2.05 [ns]
N	10	8	8	10	8	8	10	8	8	10	8	8

[y]Relative effect = [(Treatment/Control) – 1] × 100.

[z] = ** and ns denote significant differences between the treatments at $P \leq 0.05$ and not significant at $P \leq 0.10$, respectively.

Table 4.3 Soil electrical conductivity (mS/m^3) and soil pH under *Capsicum annuum* cv. Serrano and *C. frutescens* cv. Capistrano in micro-plots with and without salinity

Treatment	Soil electrical conductivity (mS/m^3)						Soil pH					
	Serrano			Capistrano			Serrano			Capistrano		
	Exp 1	Exp 2	Exp 3	Exp 1	Exp 2	Exp 3	Exp 1	Exp 2	Exp 3	Exp 1	Exp 2	Exp 3
Control	34.74	140.11	74.05	30.14	143.52	68.75	5.36	4.84	5.08	5.24	4.93	5.23
Salt	203.65	367.19	234.93	227.74	361.51	275.19	5.41	4.86	5.38	5.26	4.86	5.01
Relative effect (%)[y]	486.21	162.07	217.26	655.61	151.88	300.28	0.93	0.41	5.91	0.38	−1.42	−4.21
Std error	16.10	28.35	26.21	9.29	25.05	18.52	0.08	0.08	0.11	0.07	0.08	0.11
t-value[z]	2.03**	2.05**	2.05**	2.03**	2.05**	2.05**	2.03 ns	2.05 ns	2.05**	2.03 ns	2.05 ns	2.05**
N	10	8	8	10	8	8	10	8	8	10	8	8

[y]Relative effect = [(Treatment/Control) – 1] × 100.

[z] = ** and ns denote significant differences between the treatments at $P \leq 0.05$ and not significant at $P \leq 0.10$, respectively.

Mashela and Nthangeni (2002) proposed that the reduction of stem diameter is a mechanism through which the concentration of chlorophyll content solutes translocated to roots are being reduced in order to minimise the effects of turgor pressure in root cells. In addition to this restricted pathway, chlorophyll content solutes are also stored as starch, which is non-osmotic. In this study, NaCl + $CaCl_2$ salinity had no significant effect on dry shoot mass for both cultivars, suggesting that the two cultivars are tolerant to salinity.

The reduction of chlorophyll content by NaCl + $CaCl_2$ salinity on pepper in this study agreed with observations in another pepper study (Bethke and Drew, 1992) and those in other crops (Bongi and Loreto, 1989; Mashela and Mphosi, 1999). Bethke and Drew (1992) observed that NaCl salinity reduced chlorophyll content in *Capsicum* species and concluded that the reductions were primarily due to stomatal closure. Generally, salinity stresses result into stomatal closure (Flanangan and Jeffries, 1989).

In most cited studies (Chartzoulakis and Klapaki, 2000; De Pascale *et al*., 2000; Lee, 2006; Yildirim and Güvenc, 2006) on the influence of NaCl salinity on the biomass of various *Capsicum* species, salinity did not include Ca ions as should be in salinity studies (Bohn *et al*., 1985; Epstein, 1961). Epstein (1961) demonstrated that Ca ions were indispensable in salinity studies for the normal physiological operation of roots. Also, the selectivity of K over Na is strictly dependent upon Ca ions (Epstein, 1961). Maas (1993) demonstrated that a soil solution containing Ca is a required physiological ion *milieu* for root tissues. In most agricultural soils, where salinity problems occur, Ca ions have the

highest concentrations of all ions (Bohn *et al*., 1985; Sposito, 1989). Removal of Ca ions from root tissues using ethylenediamine tetraacetic acid (EDTA) reduced the ability of root cells to absorb and retain ions (Hanson, 1960).

Sanogo (2004) noted that the interaction between *Phytophthora capsici* and NaCl + $CaCl_2$ salinity was not significant on pepper. In this study, responses of various parameters to NaCl + $CaCl_2$ salinity were comparable to those observed on Serrano and Capistrano, which strengthen the case of including Ca ions when studying the influence of salinity in pepper production. Using NaCl salinity without including Ca ions as in various salt tolerant studies on *Capsicum* species (Chartzoulakis and Klapaki, 2000; De Pascale *et al*., 2000; Lee, 2006; Yildirim and Güvenc, 2006), did not represent the natural physiological ion *milieu* for root tissues. Apparently, the exclusion of Ca ion may explain contradicting observations between results of this study and those of others, where the observed NaCl salinity damage on pepper was severe.

Due to the increased number of ions in solutes, salinity increases soil EC (Bohn *et al*., 1985; Donahue *et al*., 1990). The changes in soil pH under salinity may be due to physiological responses in roots to salt conditions. Generally, when the root is subjected to increased stress, a large number of proteins disintegrate into the basic units, namely amino acids. These amino acids are exuded into soil solutions, resulting in reduction of soil pH. However, in this study it is difficult to discuss the observed effect in Serrano and Capistrano since the treatment increased and decreased soil pH, respectively.

In conclusion, results of this study suggested that *C. frutescens* cultivar Serrano and *C. annuum* cultivar Capistrano were tolerant to NaCl + $CaCl_2$ salinity. The two cultivars are, therefore, suitable for use in Limpopo Province, where salinity levels in irrigation water are increasing.

CHAPTER 5
RESPONSES OF TWO *CAPSICUM* CULTIVARS TO SALINITY, *MELOIDOGYNE INCOGNITA* RACE-2 AND HARVEST TIME

5.1 Introduction

The disease triangle model suggests that disease is a function of the host, pathogen and environment over time. In most host-sensitivity to pathogens and/or environment, the time factor is not taken into consideration since they are performed over a specified time instead of over a range of times.

The southern root-knot nematode (*Meloidogyne incognita*) is one of the major limiting pests in pepper (*Capsicum* spp.) production in tropical regions (Di Vito *et al*., 1985; Lindsey and Clayshulte, 1982; Thomas *et al*., 1995), particularly in areas with salinity problems. *Meloidogyne incognita* induces the formation of galls on roots, causing stunted plant growth, decreased water uptake, nutrient absorption and evapotranspiration, with increased root exudates that reduce soil pH (Bird, 1977; Lal and Yadav, 1975; Mashela, 2002; Maqbool *et al*., 1987; Sikora and Fernandez, 2005). The availability of certain essential nutrient elements is extremely sensitive to changes in soil pH (Bohn *et al*., 1985), which may provide some explanation on how this nematode affects nutrient uptake (Mashela, 2002). Nematode-resistant crop cultivars are the best alternatives to suspended synthetic nematicides in management of plant-parasitic nematodes. However, the presence of a large number of hosts to *M. incognita* and the existence of four races in this nematode, makes it difficult to develop a crop rotation plan.

Salinity has a detrimental effect on population densities of nematodes on both annual and perennial crops (Edongali *et al.*, 1982; Head and Heilman, 1971; Machmer, 1958; Mashela and Nthangeni, 2002; Mashela *et al*., 1992a; 1992b). Continuous salinity reduced hatching and infectivity of *Meloidogyne* species and *Tylenchulus semipenetrans* (Bird, 1977; Dropkin *et al*., 1958; Lal and Yadav, 1975; Mashela *et al.*, 1992; Maqbool *et al*., 1987). After a 7-day exposure of second stage juveniles (J2s) and eggs of *M. incognita* and *M. javanica* and *T. semipenetrans* to salinity, decreased egress and increased mortality were observed (Khan and Khan, 1990). Also, when subjected to continuous salinity, population levels of *Aphelenchus avenae*, *Belanolaimus longicaudatus, Hoplolaimus galeatus, Pratylenchus thornei*, *Helicotylenchus* spp., and *Rotylenchulus reniformis* showed considerable reduction (Hixson *et al*., 2005; Lal and Yadav, 1976). However, cyclic salinity, as occurs under natural conditions, increased population densities of various nematode species under both field and greenhouse conditions (Machmer, 1958; Mashela *et al.*, 1992a).

Limpopo Province, characterised by large tracts of sandy soil and tropical climate, is a vegetable-producing region for the South African fresh produce markets. The Province is renowned for its large deposits of NaCl in the Soutpansberg District and calcitic lime in Lepelle-Nkumpi District. These deposits contaminate underground sources of irrigation water. Most of the northern parts of the Province, with tropical climate and sandy soils, have high population densities of *M. incognita* race 2 (Mashela, 2002). Consequently, salt-tolerant and nematode-resistant cultivars are becoming increasingly essential because of salt-accumulation in soil and the suspension of environmental-unfriendly nematicides.

However, most salt-tolerant cultivars are not resistant to plant-parasitic nematodes, and *vice versa* (Mashela and Nthangeni, 2002). Mashela *et al*. (1992b) observed that under salinity, citrus seedlings lost their resistance to *T. semipenetrans*.

Capsicum annuum cultivar Serrano and *C. frutescens* cultivar Capistrano are commercially produced in Limpopo Province as chillies and green peppers, respectively. The two cultivars are each tolerant to NaCl + $CaCl_2$ salinity (chapter 4), whereas Serrano and Capistrano are susceptible and resistant to *M. incognita* race 2, respectively (chapter 3), over a short-time. However, the interaction of *M. incognita* infection, salinity damage and length of growing season on the productivity of the two *Capsicum* species as proposed in the disease triangle model is not documented. The objective of the study was to determine the responses of Serrano and Capistrano to NaCl + $CaCl_2$ salinity and *M. incognita* race 2 over three harvest times.

5.2 Materials and methods

Study site and plant material: The study was conducted under microplot conditions at the Horticultural Unit of the University of Limpopo (23°53”10’S, 29°44”15’E). Plots comprised 30-cm-diameter plastic pots, inserted into 28-cm-deep holes at 1.0 m x 1.0 m inter-row and intra-row spacing with a population of 8 333 plants/ha. Pots were filled with 10 ℓ steam-pasteurised Hutton soil collected from the topsoil of the dug holes, comprising 65% sand, 30% clay, 5% silt; 1.6% organic C, EC_e of 0.148 dS/m and pH 6.5. The experiments were conducted using three-week-old pepper seedlings from Martindale Seedlings CC (Tzaneen, RSA).

Preparation of experimental materials: *Meloidogyne incognita* race 2, obtained from the Agricultural Research Council, Grain Crops Institute – Potchefstroom, was cultured on tomato (*Lycopersicon esculentum*) cultivar Floradade in the greenhouse and prepared for inoculation as previously described (chapter 3). Approximately 0.92 mols NaCl + 0.32 mols $CaCl_2$ per m^3 were prepared as salt solutions (chapter 4).

Experimental design: The 2 x 2 x 3 factorial experiment was arranged in a split-split plot design with each treatment replicated 8 times. The arrangement contained nematode infection (N_o; N_1) in main plots, salinity (S_0; S_1) in sub-plots and harvest-time (90, 120, 120 days) in sub-sub-plots.

Initiation of treatments: Inoculation with nematodes was done on transplanting day to simulate natural conditions by applying ca. 8 000 J2s and eggs of *M. incognita* race 2 per pot into 10-cm-deep holes around the crown of the seedling using a 20-mℓ-plastic syringe. Salinity was initiated a day after transplanting through application of 1-ℓ salt solution (S_1) per plant, whereas control plants received similar quantities of tapwater during irrigation.

Cultural practices: Weeds and foliar pests such as aphids and whiteflies were controlled when necessary using hand-hoes and Metasystox-R (56), respectively. Irrigation was scheduled as described previously (Chapter 4). Plants were fertilised using 2.5 g of 2:3:2 N: P: K (22% active) per plant at transplanting and repeated at first fruit harvest.

Data collection: Fruits, plant height, stem diameter, chlorophyll content, dry shoot and fresh root mass, soil pH, soil EC and nematode numbers were collected at 90, 120 and 150 days after treatment. Chlorophyll content was measured from three fully-developed leaves using a hand-held chlorophyll meter between 09h00 and 10h00 a day before harvesting the experiments. Stems were cut at the crown and stem diameter measured at 10-cm distal to the severed end using digital vernier callipers. Pots were emptied to remove roots that were immersed in water to free soil particles, with excess water removed from roots and weighed. Roots were evaluated for galls and necrotic spots, cut into pieces, put in 1% NaOCl and mechanically shaken for 60 seconds. Samples were sieved through nested sieves of 150-µm and 25-µm openings, eggs plus juveniles were collected from the 25-µm-openings sieve. Approximately 250 mℓ soil samples were collected from each pot for nematode determination using the sugar-floatation extraction method (Coolen, 1979). Nematode samples were temporarily stored at 5^{o}C before nematode count under a microscope. Shoots were oven-dried at 70^{o}C for 72 hours for determination of dry shoot matter. Nematode numbers were expressed per total roots per plant and per total soil volume per plant. Soil pH and electrical conductivity (EC) were measured using a pH meter and an EC meter (Rhue and Kidder, 1983).

Data analysis: Data were subjected to analysis of variance (ANOVA) procedure using SAS software (SAS Institute, Inc., Cary, NC, 1998). Nematode numbers were transformed by Ln (x + 1) before analysis in order to homogenise the variances (Petersen, 1994), but untransformed data were reported.

5.3 Results

Reproductive factors: Salinity × time interaction was significant ($P \leq 0.05$) for the reproductive factors (RFs) under both cultivars (data not shown). At 90-day, the RFs under Serrano with and without salt were less than one, whereas at 120 and 150 days the RFs were above one. Relative to no salt treatment, the RFs under salinity had decreased by 46%, 19% and 25% at 90, 120 and 150 days, respectively (Table 5.1). Under Capistrano, the RFs were less than one at all harvests. Relative to no salt treatment, the RFs under salinity had decreased by 64%, 21% and 33% at 90, 120 and 150 days, respectively.

Table 5.1 Reproductive factors (RF) of *Meloidogyne incognita* race 2 on *Capsicum annum* cultivar Serrano and *C. frutescens* cultivar Capistrano at 90, 120 and 150 days after inoculation

Serrano						
Time		S_o		S_1		Relative effect
(days)	Pi	Pf	RF	Pf	RF	within time[z]
90	8 000	5 032	0.63	2 711	0.34	−46%
120	8 000	19 330	2.42	15 735	1.97	−19%
150	8 000	28 090	3.51	21 192	2.65	−25%
Standard error = 0.67						
Capistrano						
90	8 000	2 856	0.36	2 950	0.37	−64%
120	8 000	4 954	0.62	3 889	0.49	−21%
150	8 000	7 490	0.94	5 075	0.63	−33%
Standard error = 1.22						

[z]Relative effect within time (%) = (RF with salt/RF without salt −1) × 100

Pepper productivity: All treatments had no effect on variables measured in Capistrano cultivar (Data not shown). However, nematode × time interactions in Serrano were significant ($P \leq 0.05$) on six variables measured, whereas none of the second-order

interactions were significant (Table 5.2). The variables with first-order interactions were further analysed using a two-way table (Petersen, 1994).

Fresh fruit yield and dry shoot mass: Relative to nematode-free treatment, nematode infection increased fresh fruit yield of Serrano by 10% at 90 days, but reduced this variable by 28% and 12% at 120 and 150 days, respectively (Table 5.3). In contrast, nematodes increased dry shoot mass by 6% and 3% at 90 and 120 days, respectively, but reduced this parameter by 44% at 150 days.

Stem diameter and fresh root mass: Relative to nematode-free treatment, nematodes reduced stem diameter of Serrano by 2% and 23% at 90 and 150 days, respectively, but increased the parameter by 1% at 120 days. However, fresh root mass increased by 10%, 13% and 38% at 90, 120 and 150 days, respectively.

Chlorophyll content and soil pH: Relative to nematode-free plots, nematodes increased chlorophyll content in Serrano by 8% and 9% at 90 and 150 days, respectively, but reduced the parameter by 8% at 120 days (Table 5.5). Relative to salt-free treatment, salinity reduced soil pH by 1%, 10% and 14% at 90, 120 and 150 days, respectively.

Soil EC_e: Relative to nematode-free control without salt, nematode infection with and without salinity increased soil EC by 17% and 6%, respectively, under Serrano production (Table 5.6).

Table 5.2 Partial ANOVA of a split-split plot arrangement of two levels of *Meloidogyne incognita* race 2, two levels of salinity and three levels of harvest-times on fresh fruit mass, dry shoot mass, plant height, stem diameter, root mass and chlorophyll content of *Capsicum frutescens* cultivar Serrano.

Source	DF	Fresh fruit wt		Dry shoot wt		Plant height		Stem diameter		Root wt		Chlorophyll content	
		MS	F	MS	F	MS	F	MS	F	MS	F	MS	F
Nematode (N)	1	12.80	4.42^{**}	1134.03	23.36^{***}	2625.04	18.70^{***}	634.28	6.46^{**}	9472.43	13.75^{***}	101.60	2.55^{ns}
Rep (R)	7	7.57	2.61^{**}	186.97	3.85^{***}	258.94	1.84^{*}	205.74	2.09^{*}	2846.05	4.13^{***}	67.71	1.70^{ns}
Error a	7	3.67	-	75.73	-	178.37	-	115.01	-	1189.77	-	127.10	-
Salinity (S)	1	25.59	8.84^{***}	6.91	0.14^{ns}	388.74	2.77^{ns}	438.87	4.47^{**}	4123.36	5.99^{**}	10.22	0.26^{ns}
S × N	1	4.56	1.57^{ns}	55.37	1.14^{ns}	233.69	1.66^{ns}	50.23	0.51^{ns}	1342.21	1.95^{ns}	108.46	2.73^{ns}
Error b	14	4.57	-	112.20	-	229.28	-	44.55	-	1436.90	-	36.29	-
Time (T)	2	71.83	24.81^{***}	1511.16	31.12^{***}	2860.90	20.38^{***}	850.98	8.66^{***}	10918.90	15.85^{***}	705.00	17.72^{***}
N × T	2	13.49	4.66^{**}	963.81	19.85^{***}	1185.34	8.44^{***}	409.95	4.17^{**}	9759.88	14.17^{***}	232.06	5.83^{***}
S × T	2	5.09	1.75^{ns}	77.48	1.60^{ns}	419.30	2.99^{*}	260.89	2.66^{*}	1756.24	2.55^{*}	52.25	1.31^{ns}
N × S × T	2	3.20	1.11^{ns}	83.77	1.73^{ns}	75.84	0.54^{ns}	89.57	0.91^{ns}	1143.00	1.66^{ns}	13.18	0.33^{ns}
Error c	56	2.90	-	48.55	-	140.41	-	98.22	-	688.72	-	39.78	-

Table 5.3 Relative effect of infection with *Meloidogyne incognita* race 2 on fresh fruit yield and dry shoot mass of *Capsicum frutescens* cultivar Serrano over 90, 120 and 150 days in micro-plots

Time (days)	Fresh fruit yield (g)			Dry shoot mass (g)		
	Nematode					
	N_o	N_1	Relative effect[z]	N_o	N_1	Relative effect
90	201.33	221.45	10%	19.89	21.12	6%
120	200.76	144.27	−28%	22.79	23.37	3%
150	84.58	74.80	−12%	33.50	18.70	−44%
	Standard error = 9.88			Standard error = 1.11		

[z]Relative effect within time = $[(N_1/N_o - 1) \times 100]$

Table 5.4 Relative effect of infection with *Meloidogyne incognita* race 2 on stem diameter and Fresh root mass of *Capsicum frutescens* cultivar Serrano cultivar over 90, 120 and 150 days in micro-plots

Time (days)	Stem diameter (mm)			Fresh root mass (g)		
	Nematode					
	N_o	N_1	Relative effect[z]	N_o	N_1	Relative effect
90	13.35	13.06	−2%	30.01	33.09	10%
120	10.85	10.96	1%	39.77	44.80	13%
150	12.88	9.98	−23%	50.23	69.50	38%
	Standard error = 0.22			Standard error = 1.43		

[z]Relative effect within time = $[(N_1/N_o - 1) \times 100]$

Table 5.5 Relative effect of infection with *Meloidogyne incognita* race 2 on chlorophyll content and relative effect of irrigating with salt solutions on soil pH under *Capsicum frutescens* cultivar Serrano over 90, 120 and 150 days in micro-plots

Time (days)	Chlorophyll content			Soil pH		
	Nematode			Salt		
	N_o	N_1	Relative effect[z]	S_0	S_1	Relative effect
90	65.28	70.68	8%	5.38	5.30	−1%
120	67.74	62.38	−8%	5.36	4.84	−10%
150	55.61	60.60	9%	5.12	4.40	−14%
	Standard error = 1.12			Standard error = 1.63		

[z]Relative effect (%) = [(Treatment/Control) – 1] × 100

Table 5.6 Interaction effect of *Meloidogyne incognita* race 2 and salinity on soil EC of *Capsicum frutescens* cultivar Serrano in micro-plots (Standard error = 12.55)

Salt	Nematode		
	N_o	N_1	Relative effect[z]
S_o	78.47	83.13	6%
S_1	265.21	311.07	17%

[z]Relative effect (%) = [(Treatment/Control) – 1] × 10

5.4 Discussion

Nematode responses to salt x harvest time: The less than unity RFs at all harvest times in Capistrano under both salinity and non-salinity confirmed previous observations under non-saline conditions (Chapter 3), that this cultivar was a non-host to *M. incognita* race 2. The reductions of the RFs under NaCl + $CaCl_2$ salinity confirmed previous observations where salinity suppressed nematode numbers of *T. semipenetrans, A. avenae, B. longicaudatus, H. galeatus, P. thornei, Helicotylenchus* spp., and *R. reniformis* (Hixson *et al*., 2005; Lal and Yadav, 1976; Mashela *et al*., 1992a). Generally, salinity reduced nematode numbers by suppressing egg hatch, nematode development and infection and by increasing juvenile mortality (Dropkin *et al*., 1958; Edongali *et al*., 1982; Khan and Khan, 1990; Lal and Yadav, 1975; Mashela *et al*., 1992a; 1992b; Maqbool *et al*., 1987)

Under field conditions Machmer (1958) observed that application of salt increased population numbers of various nematodes. Mashela *et al.* (1992a) demonstrated cyclic salinity increased population numbers of nematodes, whereas continuous salinity decreased the numbers of nematodes.

Nematode infection predisposes plants to salinity damage (Heald and Heilman, 1971; O'Bannon and Esser, 1985). However, all variables measured in Capistrano cultivar did not respond to any of the three main factors. Studies in South America suggested that Capistrano pepper cultivar was sensitive to *M. incognita*, without specifying the *M. incognita* race which was being studied (Thomas *et al*., 1995). *Meloidogyne incognita* has four races, namely, *M. incognita* race 1, *M. incognita* race 2, *M. incognita* race 3 and *M. incognita* race 4 (Hartman and Sasser, 1985). Nematode races

within the same species such as *M. incognita* are morphologically similar and can generally be distinguished through differential hosts (Hartman and Sasser, 1985) and molecular markers (De Waele and Elsen, 2007). The most predominant *M. incognita* races in South America are race 1 and race 3 (Di Vito *et al*., 1995; Lindsey and Clayshulte, 1982; Thomas *et al*., 1995). Generally, in that part of the world, when the race is not specified in studies that involve *M. incognita*, one may infer that either *M. incognita* race 1 or race 3 was involved. In the contrary, the predominant race in Limpopo Province, South Africa, is *M. incognita* race 2 (Mashela, 2002).

In Capistrano cultivar, all interactions among any of the three factors were not significant ($P \leq 0.05$). The factorial experiment, when the interactions are not significant ($P \leq 0.05$), demonstrates that two or more factors have either an additive or a multiplicative effect (Salisbury and Ross, 2000). In additive responses, the two factors act on two different sites or organelles to elicit a greater response, whereas in multiplicative responses, the two factors act on different steps of a process in the same causal sequence, so that the effect of one is always a fraction of the other. However, since the main factors were also not significant in this cultivar, this suggests that Capistrano cultivar is strongly resistant to *M. incognita* and tolerant to NaCl + $CaCl_2$ salinity, regardless of the time-frame. In contrast, most interactions were significant ($P \leq 0.05$) on various parameters measured in the productivity of Serrano pepper cultivar. When the interaction of two or more factors is significant ($P \leq 0.05$), the interaction is said to be synergistic (Salisbury and Ross, 2000), which implies that the factors are acting on the same site, resulting in a much greater response than when the reaction towards their action is the sum of individual reactions (Salisbury and Ross,

2000). Thus, in Serrano cultivar, various factors acted on the same site or organelle to elicit a greater response, and these interactions are discussed further in this study.

Fruit yield responses to nematode × harvest time: The 10% increase in fruit yield in Serrano-infected with nematodes at 90 days is one of the interesting features in plant-parasitic nematodes, which may be better understood through using the concept of Seinhort's (1965) damage threshold levels. By definition, the damage threshold level is that level of nematode numbers where yield reduction starts. Before the damage threshold, infection by various nematode species invariably increases plant yield (Coursen and Jenkins, 1958; Seinhorst, 1965; Wallace, 1973). The mechanism involved in the increase of yield under infection of plants by low nematode numbers is not clear. Milne *et al*. (1977) demonstrated that improved growth responses to fenamiphos application in pineapple were in part due to increased accumulation of indole-acetic acid in plant tissues, which stimulates plant growth. The observed increase in fruit yield in response to low nematode numbers is the first such observation in *C. frutescens* cultivar Serrano. However, similar increases were observed in tomato infected with low numbers of *M. incognita* race 2 (Mashela, 2002; Mashela and Nthangeni, 2002; Mashela *et al*., 2008). The reduction in fresh fruit yields at 120 and 150 days after inoculation with nematodes suggested that nematode numbers were then beyond the Seinhorst (1967) damage threshold levels. Since infection by *M. incognita* race 2 at 120 and 150 days had consistently reduced fruit yield in Serrano, this nematode race may be a limiting economic factor when this cultivar is grown for an extended period in regions like Limpopo Province, where the race thrives.

Dry shoot mass responses to nematode × harvest time: The increase in dry shoot mass of Serrano in all harvest dates except at 150 days under *M. incognita* race 2 can be explained using the damage threshold levels, as previously done for fruit production. Generally, *M. incognita* infection reduces dry shoot mass in various crops (Thomas *et al.*, 1995). In South America, *M. incognita* races 1 and 3 are, as measured through their negative effect on dry shoot mass, economic pests of pepper (Thomas *et al.*, 1995), which agreed with current results at 150 days.

Stem diameter responses to nematode × harvest time: Notably at 90 and 150 days, the stem diameter of Serrano decreased under nematode infection. Infection by *Meloidogyne* species invariably results in reduction in stem diameter (Mashela, 2002; Mphosi, 2004). The decrease in stem diameter has also been observed in plants with arbo-vascular mycorhiza-inoculated roots (Graham and Syvertsen, 1989), with plants growing under salinity stress (Mashela and Nthangeni, 2002), those subjected to drought stress (Mafeo, 2005) and those under root pruning (Mashela and Nthangeni, 2002). Mashela and Nthangeni (2002), in their stem girdling, root-pruning and *T. semipenetrans* study, explained the decrease in stem diameter as a physiological response to curb turgor pressure in affected cells. In their model, Mashela and Nthangeni (2002) demonstrated that accumulation of carbohydrates in an organ decreased osmoticum ions (K, Na, Cl), whereas reduction of carbohydrates had an opposite effect.

Fresh root mass responses to nematode × harvest time: Generally, root mass in *Meloidogyne*-infected susceptible plants increases due to the development of root-galls (Dropkin, 1980; Hartman and Sasser, 1985), which was confirmed in Serrano.

Chlorophyll content responses to nematode × harvest time: Generally, nematode-infection reduced chlorophyll content in Serrano. This confirmed previously documented work in chlorophyll content of citrus seedlings under *T. semipenetrans* infection (Mashela and Mphosi, 1999).

Soil pH responses to salt × harvest time: The decrease of soil pH under salinity is commonly documented (Bohn *et al*., 1985). Generally, *M. incognita* interacts with salinity to reduce soil pH (Mashela, 2002; Mphosi, 2004). Infection of roots by *Meloidogyne* species results in the release of various amino acids from the root galls (Wallace, 1973), which invariably reduces soil pH. The observed negligible effect of *M. incognita* race 2 infection of Serrano on soil pH may suggest that tolerant species have a different chemical behaviour with respect to accumulation of amino acids in stressed root cells.

Soil EC responses to nematode × salt: Salinity alone increases soil EC (Bohn *et al*., 1985), whereas infection with high levels *M. incognita* race 2 on tomato also consistently increased soil EC (Mashela, 2002; Mashela and Nthangeni, 2002; Mphosi, 2004; Ngobeni, 2003). The significant interaction between these two factors on EC implies that they increase the soil EC through acting on the same factor, which was previously thought to be organic acids that dissociate into OH^- and H^+ ions (Mashela, 2002).

In conclusion, results of this study suggested that *C. annuum* cultivar Capistrano was resistant to *M. incognita* race 2 and it was also tolerant to salinity. Also, *M. incognita*

× harvest time interaction and salt × harvest time interactions acted synergistically on Serrano pepper cultivar, whereas the three-factor interactions were either additive or multiplicative on variables measured in Capistrano. Results of this study suggested that Serrano was highly susceptible to *M. incognita* race 2 over time, but was moderately tolerant to low levels of NaCl + $CaCl_2$ salinity. Therefore, this cultivar is not suitable for use in crop rotation programmes for suppression of *M. incognita* race 2 in areas with and without salinity, whereas Capistrano is suitable.

CHAPTER 6
SUMMARY AND CONCLUSIONS

6.1 Summary of the study

The study evaluated three objectives: (1) host-status and host-sensitivity of *Capsicum frutescens* cultivar Serrano and *C. annuum* cultivar Capistrano to the root-knot nematode *(Meloidogyne incognita* race 2), (2) responses of Serrano and Capistrano to salinity, and (3) interactive responses of Serrano and Capistrano to *M. incognita* race 2, salinity and three harvest times. All experiments were conducted under microplot conditions.

6.1.1 Host-status

Serrano (*Capsicum frutescens*) was sensitive to *M. incognita* race 2, that is, it allowed reproduction of this race and in turn the race reduced its productivity. In contrast, Capistrano (*Capsicum annuum*) was resistant to *M. incognita* race 2 because it did not allow the nematode to reproduce nor did it suffer any damage due to infection with the race.

6.1.2 Salt-status

Salinity had no effect on numbers of *M. incognita* race 2 under both Serrano and Capistrano at 120 days after the initiation of the salt treatment. Also, both Serrano and Capistrano appeared to be tolerant to NaCl + $CaCl_2$ salinity at the salt level used in this study. The increase in soil EC under salt treatment in both cultivars suggested that the salt treatment was effective in changing the salinity of the rhizosphere. Salinity results in this study were not comparable with the results of salinity in other salt-tolerant studies on pepper cultivars, since in those studies Ca was not included in the

induction of salinity by NaCl. Epstein (1961) demonstrated that Ca was indispensable in selective and K transport by plant cells. All NaCl salinity studies in agricultural crops included $CaCl_2$ (Epstein, 1972; Graham and Syvertsen, 1989; Mashela, 1992; Mashela and Nthangeni, 2002).

6.1.3 Nematode-salinity interaction over time

Capistrano did not respond to any of the interactions due to salinity, time and *M. incognita* race 2. Serrano responded negatively to various two-factor interactions between salinity, time or *M. incognita* race 2.

6.2 Conclusions

Results of this study suggested that Capistrano pepper cultivar was resistant to *M. incognita* race 2, whereas Serrano was sensitive to infection by this nematode race. Both pepper cultivars were salt tolerant. Consequently, both cultivars can be used in areas with salinity problems, whereas Capistrano can be used in areas with *M. incognita* race 2 in a crop rotation programme.

6.4 Recommendations

In future studies, the mechanisms involved in resistance of Capistrano to *M. incognita* race 2 should be established in order to determine whether the resistance is sustainable or not. Field surveys to substantiate the existence of this pathogen in commercial pepper producing areas of Limpopo Province would strengthen the crop rotation plan proposed in this study.

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