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Southern Sting Nematode (*Ibipora lolii*) on Turf Grass in Western Australia

Peter Edwin Ruscoe

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Abstract

Since being identified in the late 1970s as a damaging pest of turf grass in Australia, there has been very limited scientific research on the southern sting nematode (*Ibipora lolii)*. The aims of this study were to better understand its distribution and population dynamics in the Perth region of Western Australia, and to investigate the resistance and tolerance of turf cultivars as a potential management strategy.

In a survey conducted on municipal sports fields in 2015, *I. lolii* was detected in 51% (46 out of 90) randomly-selected sites from 21 council areas. In the sites where it was detected, the mean population density was 27 nematodes/200 mL of soil. However, it was observed that infestations were not evenly distributed within the sports fields. This was based on the presence of symptoms, such as sparse coverage and shallow root systems, in the predominantly kikuyu turf grass. Further testing of ten selected sites in 2016 found the mean number of *I. lolii* was much higher (202 nematodes/200 mL of soil) in localised areas where the turf was in poor condition. These were often located in heavily-trafficked areas where the original turf had been replaced with planting material from turf farms that were infested with the nematode.

A 2-year study (2016 to 2018) of population dynamics at two kikuyu sports fields found that *I. lolii* exhibited a pattern of seasonal fluctuation in population density. Total numbers increased in cool and wet conditions from May to October, and declined during the hot and dry period from November to April. Juvenile stages were present at every sampling date, indicating that *I. lolii* reproduces all-year-round, peaking in October. In terms of distribution down the soil profile, the highest population density of *I. lolii* was in the top 10 cm, where the majority of the root system is located. However, the nematode was found at all sampling depths to 70 cm, demonstrating that it is also capable of deep vertical movement.

A field experiment was established in 2017 to evaluate two cultivars of couch (*Cynodon dactylon* cv. Grand Prix and Wintergreen), two cultivars of hybrid couch (*C. dactylon x C. transvaalensis* cv. TifTurf and TifSport) and two cultivars of kikuyu (*Pennisetum clandestinum* cv. Village Green and common) for their resistance and tolerance to *I. lolii* in microplots infested with the nematode. There was a rapid increase in the population of *I. lolii* on all cultivars in the first 2 months after planting, causing a severe decline in the condition of the turf. After 4 months, there were no statistically significant differences in nematode

populations, indicating that none of the cultivars were resistant to *I. lolii*. Also, none of the cultivars were able to tolerate feeding by the nematode, as the infested microplots had an overall average of 88% less root growth than the adjacent non-infested microplots after four months. Nevertheless, couch cultivars Grand Prix and TifTuf tended to maintain better surface integrity than the other couch and kikuyu cultivars infested with *I. lolii*, although further investigation is warranted to evaluate their performance over a much longer period.

Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

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No publications

Publications included in this thesis

No publications included

Contributions by others to the thesis

Graham Stirling (Biological Crop Protection Pty. Ltd.) has made a significant contribution to the conception and design of this research. He has also provided the necessary training and technical support to undertake this study.

Ken Johnston (Sports Turf Technology Pty. Ltd.) has collaborated on the design of the field experiment and made a significant contribution to its establishment. He has also assisted with all of the nematode extraction work undertaken in this project.

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Statement of parts of the thesis submitted to qualify for the award of another degree

None

Research Involving Human or Animal Subjects

No animal or human subjects were involved in this research.

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nematodes, turf grass, plant pathology

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Chapter 1. Introduction

Turf grasses can host a range of plant-parasitic nematodes. These nematodes are obligate parasites, described as transparent, microscopic roundworms (mostly 0.5-2 mm long). They feed on the root system of turf using a well-developed stylet, or spear-like mouthpart that penetrates plant cells. This can cause damage to roots, such as swelling and girdling that leads to poor root function, or root pruning that results in a shallow or stubby root system. The effects of nematode feeding generally become apparent only when conditions become unfavourable for the growth of turf. The visible above-ground symptoms mimic symptoms of stress e.g. wilting, chlorosis, lack of vigour, and are likely to be most evident under stressful conditions (Smiley *et al*. 2005).

The southern sting nematode *(Ibipora lolii)* was first described by Siviour and McLeod (1979) in turf grass with very poor root growth and unthrifty appearance on bowling greens and golf courses in the Newcastle region of NSW. Stirling *et al.* (2013) used molecular evidence to differentiate it from the North American sting nematode, *Belonolaimus longicaudatus*, and show that *I. lolii* is not an Australian native species.

There is strong evidence to indicate that *I. lolii* was introduced to the Perth metropolitan area of Western Australia during the 1970s on turf planting material from Newcastle. It has since become a serious problem on irrigated turf grass within the region known as the Swan Coastal Plain. This is a narrow strip of land (15-30 km wide) between the Darling Range and Indian Ocean that is predominantly composed of a series of coastal sand dune systems. The sandy soils of the Swan Coastal Plain provide an ideal habitat for this nematode (Stirling *et al.* 2013). Examples of the damage it causes to turf grass can be seen in Figs. 1.1 and 1.2.

B. longicaudatus has been studied extensively in the USA, due to its economic importance on turf grass and a range of horticultural crops. However, there has been very little research into *I. lolii* in Australia. The aim of this study was to contribute to this limited knowledge base by investigating the distribution, population dynamics and management of *I. lolii* on turf grass in Western Australia.

Figure 1.1. An irregular patchwork of bare areas in kikuyu, the most common turf grass in the Perth region, infested with *I. lolii* (Photo K. Johnston).

Figure 1.2. Kikuyu with short, stubby and dysfunctional roots caused by *I. lolii* feeding on the root tips (Photo G. Stirling).

Chapter 2. Literature Review

2.1 INTRODUCTION

The sting nematode *(Belonolaimus longicaudatus)* is among the most destructive plant-parasitic nematodes in the United States, affecting a wide range of plants including turf grasses (Crow 2015). Even at relatively low population densities there is a high risk of turf damage from this nematode (Crow 2014). It is native to the sandy coastal soils of the south-eastern USA, but has become more widespread throughout the mid-west and west coast, especially with the prevalence of sand-based turf root zones (Crow 2015). *B. longicaudatus* has been studied extensively in the USA, due to its economic importance on turf grass and a range of horticultural crops.

In Australia, there is a very similar nematode, identified as *Ibipora lolii*, reported to be a widespread and destructive pest of turf grasses in the Newcastle area of New South Wales and the Perth region of Western Australia (Siviour & McLeod 1979; Stirling *et al*. 2013). It was given the common name of southern sting nematode by Stirling *et al*. (2013) to differentiate it from the North American sting nematode. In contrast to *B. longicaudatus*, there has been very limited study of *I. lolii*, with only two peer-reviewed papers having been published in the scientific literature.

This literature review will cover the taxonomy, ecology, pathogenicity and management of *B. longicaudatus,* and what little is known about *I. lolii*. It will conclude with an appraisal of what can be learnt from the existing knowledge of *B. longicaudatus* to guide further study of *I. lolii*.

2.2 TAXONOMY

2.2.1 Taxonomic position

The genera *Belonolaimus* Steiner, 1949, from North America, and *Ibipora* Monteiro & Lordello, 1977, from Central and South America, belong to the nematode family Belonolaimidae and sub-family Belonolaiminae Whitehead, 1960. This sub-family also includes the Australian native genus *Morulaimus* Sauer, 1966. These are all moderate to large sized nematodes that feed exclusively as ectoparasites, using a long, slender stylet to penetrate root cells (Fortuner & Luc 1987; Stirling *et al*. 2013).

Ibipora was proposed as a new genus by Monteiro & Lordello (1977) because it was an intermediate between *Belonolaimus* and *Morulaimus*, combining the characteristics of both genera. It possesses a lip region similar to *Belonolaimus*, but has a lateral field with four longitudinal lines, as in *Morulaimus*.

In their description of the new species *I. lolii*, Siviour & McLeod (1979) placed it in *Ibipora* on the basis of the four lines in the lateral field. They recognised that the genera *Belonolaimus* and *Ibipora* were separated solely on the number of lines in the lateral field, one in *Belonolaimus* and four in *Ibipora*. It was also acknowledged that additional separating characters were needed to consolidate *Ibipora* as a distinct genus.

Fortuner & Luc (1987) reappraised the sub-family Belonolaiminae and proposed that *Ibipora* was a junior synonym of *Belonolaimus*, effectively transferring all of the species in *Ibipora* back to *Belonolaimus*. They considered that the number of lateral field lines was not diagnostic at generic level. While acknowledging the difference in stylet length between the species in *Belonolaimus* and *Ibipora*, their conclusion was that this did not warrant the recognition of a separate genus.

In 1992, Nobbs & Eyres described a new species, *Morulaimus gigas*, collected from turf in Western Australia. According to Stirling *et al*. (2013), the morphological description and measurements of this nematode were similar to *I. lolii* from New South Wales described by Siviour & McLeod in 1979.

Stirling *et al*. (2013) used molecular evidence to show that the nematodes originally described as *I. lolii* and *M. gigas* are identical. With *I. lolii* having priority, *M. gigas* is considered a junior synonym. The molecular sequence data presented in their study also demonstrated that *I. lolii* is different to *B. longicaudatus* and other species of *Morulaimus*. However, the authors acknowledged that in the absence of molecular data on other species of *Ibipora*, the decision to place *I. lolii* in that genus is provisional.

In order to strengthen the position of *I. lolii* in that genus, Stirling *et al.* (2013) recommended further investigation to obtain specimens of different species of *Ibipora* from Central and South America for molecular sequencing.

2.2.2 Morphological features and identification

Siviour & McLeod (1979) described *I. lolii* as having a long and slender body, with males being shorter and thinner than females. The adult nematodes ranged in length from 1.75 to 2.65 mm (mean 2.11) for males, and 2.0 to 2.6 mm (mean 2.34) for females. The female has a broadly rounded tail, as do the juvenile stages, while the male is distinguished by a tail that tapers to a sharp point and is enveloped by a long and narrow bursa. The female's vulva position is approximately half way along the body. *I. lolii* has a long and flexible stylet for feeding, the length of which is between 110 and 120 microns in adults, with flattened round knobs at the base of the stylet. Another feature of *I. lolii* is the strong median oesophageal bulb with prominent crescentric valves, which are clearly visible at a magnification of 40X. The junction of the oesophagus and intestine is obscure. Refer to the illustrations in Figure 2.1.

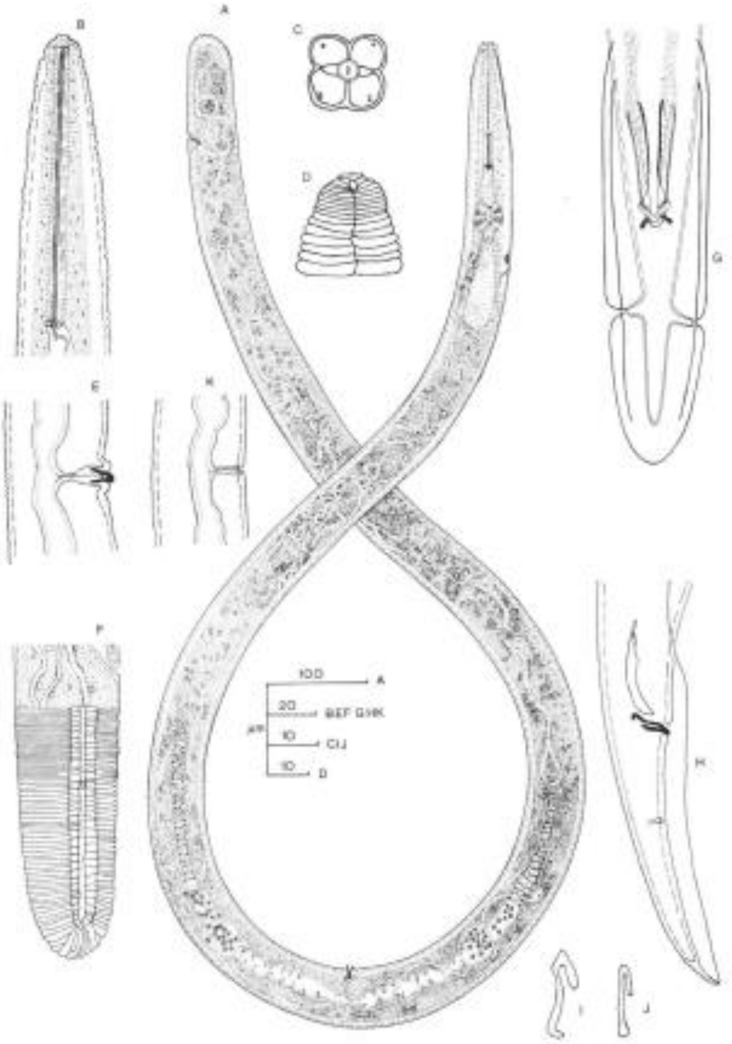

Fig. 1. *Ibipora lolii*. A. Female; B. Anterior of female; lateral; C. Face view of head; D, Head, lateral; E. Vulval region, lateral; F. Female tail, lateral; G. Male tail, ventral; H. Male tail, lateral; I. Gubernaculum, lateral, *I. lineatus*; J. Gubernaculum, lateral; K. Vulval region, lateral.

Figure 2.1. Illustrations of *Ibipora lolii* by Siviour & McLeod (1979)

Measurements of *B. longicaudatus* recorded by Rau (1961) indicate that it is slightly larger than *I. lolii*, having a body length of up to 3 mm and a stylet length of up to 140 microns. *B. longicaudatus* also has an oesophageal overlap of the intestine (Rau 1958). Siviour & McLeod (1979) identified some other minor differences between *B. longicaudatus* and *I. lolii*: the shape of the oral aperture is oval in *I. lolii* and circular in *B. longicaudatus*; and the position of the amphid openings is behind the lateral lobes of the lip region in *I. lolii* and on these lobes in *B. longicaudatus*.

2.3 ECOLOGY

2.3.1 Life cycle

The life cycle of *B. longicaudatus* was studied *in vitro* by Huang & Becker (1999). To facilitate this, a gnotobiotic culture of *B. longicaudatus* was established using the method described by the same authors in 1997. This process involved inoculating excised corn roots supported by Gamborg's B5 medium in 1.5% agar, with nematodes that had been surface-decontaminated after migrating at least 2 cm through 1.0% agar under aseptic conditions. The culture was maintained in darkness at 28°C.

In the conditions described above, Huang & Becker (1999) reported that the life cycle of *B. longicaudatus* was completed in 24 days. The first-stage juvenile (J1) appeared in the egg at 3 to 4 days after egg deposition. There was one moult in the egg and it hatched as a second-stage juvenile (J2) in another 2 days. The J2 fed for 1 to 2 days and then became immobile and underwent the second moult, which lasted for 2 days. During this moulting period, the stylet shaft and median bulb became invisible. The third-stage juvenile (J3) fed for 3 days and then entered the third moulting period at 7 days after hatching. The fourth-stage juvenile (J4) emerged after 2 days and fed for another 4 to 5 days. For juveniles that developed into males, the fourth moult began at 13 days after hatching, and the males emerged after a 2-day moulting period. The females took slightly longer to develop, with the fourth moult starting at 14 days after hatching and lasting for 3 days. The females began mating almost immediately. After mating, both the males and females fed, but the female stopped feeding before laying its first eggs at 19 days after hatching. The J2 of the second generation hatched 5 days after egg deposition, a total of 24 days after the first-generation hatching.

2.3.2 Population dynamics

The population dynamics of *B. longicaudatus* were studied on golf course fairways in California by Bekal & Becker (2000a). The primary turf species was couch/bermudagrass *(Cynodon dactylon)* that was overseeded with perennial ryegrass *(Lolium perenne)* in autumn. The population densities were monitored in the top 30 cm of soil at monthly intervals for a period of one year, on three golf courses. The conclusion from this study was that the population dynamics of *B. longicaudatus* were correlated to soil temperature for most of the year. The highest population densities occurred in warm soil conditions (>20°C) in the summer or autumn months. However, these peaks in population density were followed by rapid declines, when it was observed that damage to the turf root system by the nematode became so severe that there was probably a limited food supply. Overseeding with ryegrass caused a temporary build-up in numbers during autumn, but this was followed by a decline in the winter months. The top 15 cm of the root zone generally had higher population density than at 15 to 30 cm depth, except in the hotter months, probably indicating migration deeper in the profile. Juvenile numbers fluctuated throughout the year, with a series of peaks at time intervals that perhaps represented three generation cycles between each peak.

2.3.3 Host range

Bekal & Becker (2000b) conducted a major host range study of *B. longicaudatus* on turf grasses grown in pots. The turf species that were found to be good hosts included couch, zoysiagrass *(Zoysia spp.)*, Kentucky bluegrass *(Poa pratensis)*, perennial ryegrass, creeping bentgrass *(Agrostis stolonifera)* and tall fescue *(Festuca arundinacea)*. The increases in population of *B. longicaudatus* ranged from 9 to 43 times the initial number in 7 weeks, with zoysiagrass having the lowest increase and ryegrass the highest. Weeds including wintergrass *(Poa annua)* and crabgrass (*Digitaria sanguinalis)* were also rated as good hosts for *B. longicaudatus*.

In a field study, Pang *et al*. (2011) investigated population densities of *B. longicaudatus* in three cultivars of seashore paspalum *(Paspalum vaginatum)* and eight cultivars of couch (*Cynodon* spp.). Their conclusion was that overall, couch was a better host for this nematode than seashore paspalum. Out of the couch cultivars, TifSport had the lowest population density of *B. longicaudatus*. The population decline in TifSport over the course of the two-year study indicated that it might have some level of resistance. SeaDwarf seashore paspalum seemed to be relatively tolerant of *B. longicaudatus* as well, based on its ability to maintain greater root length and more green cover than the other seashore paspalum cultivars. This study also found that the cultivars of couch and seashore paspalum that were poor hosts for *B. longicaudatus* had the highest population densities of the spiral nematode, *Helicotylenchus pseudorobustus*.

Another turf species that is reported to be susceptible to *B. longicaudatus* is Buffalo/St. Augustinegrass (*Stenotaphrum secundatum*). In a study by Busey *et al*. (1991), the four diploid genotypes tested were good hosts, although with varying levels of turf damage. Busey *et al*. (1993) then demonstrated host resistance in polyploid genotypes. Root architecture was suggested as a possible basis for resistance, because the polyploid genotypes tend to have thicker primary roots than diploids.

Siviour & McLeod (1979) undertook a host range study of *I. lolii* on some common turf grass species grown in pots. In this study, *I. lolii* reproduced on couch, perennial ryegrass, bentgrass and Kentucky bluegrass. The largest population increase was on ryegrass, with approximately 25 times the initial number after 90 days.

Stirling *et al*. (2013) used a pot study to compare kikuyu (*Pennisetum clandestinum*) and hybrid couch (*C. dactylon* x *C. transvaalensis* cv. Tifgreen 328) as hosts for *I. lolii*. After 20 weeks, the population increase was far greater in kikuyu, with nearly 15 times the initial population density compared with a two-fold increase in couch. Sugarcane was also found to be an excellent host for *I. lolii*, indicating that it is probably capable of living on a range of grass species.

2.3.4 Distribution

Robbins & Barker (1974) investigated the ecological factors responsible for the limited distribution of *B. longicaudatus* in the USA. Their study identified that soil type is the primary limiting factor influencing its geographic distribution. The reproduction of *B. longicaudatus* was limited to soils with more than 80% sand and less than 10% clay. Being a relatively large nematode, up to 3 mm long, it relies on movement through the soil pore spaces for reproduction. In their description of *I. lolii*, Siviour & McLeod (1979) commented that it is equivalent in size to *B. longicaudatus*. This would suggest that *I. lolii* also requires soil types with high sand content. The prevalence of sand-based construction for sports turf provides an ideal habitat for these nematodes, with the potential for widening their distribution to locations where the natural soil type may be otherwise unsuitable.

Stirling *et al*. (2013) made the observation that *I. lolii* has a reasonably distinct geographic distribution pattern within New South Wales. It is mostly found in a coastal strip centred on Newcastle, between Gosford and Taree, as well as in the south-eastern coastal suburbs of Sydney. They also presented strong evidence that *I. lolii* was introduced to the Perth metropolitan area of Western Australia in turf planting material from Newcastle. It has since become widespread in turf within the region known as the Swan Coastal Plain. Nambiar *et al*. (2010) reported that *M. gigas* (=*I. lolii*) had also been detected in South Australia, Victoria and Queensland.

Human activity is responsible for spreading nematodes long distances. For example, *I. lolii* was transferred ≈3300 km from the east to west coast of Australia, and *B. longicaudatus* was introduced to southern California from the south-eastern states of the USA. The nematodes are primarily carried on infested planting material i.e. turf rolls or shredded turf containing soil and roots. Therefore, infested turf farms have the potential to spread the nematode quite rapidly. This was noted by Stirling *et al.* (2013) as a contributing factor to an exponential increase in the infested area in Western Australia.

Once introduced to a new location, the nematodes are not capable of moving very far, unless they are spread by surface water runoff or by the transfer of infested soil or turf (Smiley *et al*. 2005). While plant-parasitic nematodes are motile through the soil, the overall distance travelled under their own power probably does not exceed a metre per season (Agrios 2012). However, the lateral movement through soil of *B. longicaudatus* and *I. lolii* has not been studied.

2.4 PATHOGENICITY

2.4.1 Feeding habits

A detailed study of the feeding habits of *B. longicaudatus* was undertaken by Huang & Becker (1997), using the *in-vitro* culture method previously described in the life-cycle study (2.3.1). They found that *B. longicaudatus* fed strictly as an ectoparasite at or near the root tips, with only the stylet inserted into the root tissues. It did not feed along root sides, except on newly initiated lateral buds, demonstrating a preference for the region of cell division or elongation. The study concluded that *B. longicaudatus* needs to initiate feeding many times during its life cycle, especially for females. This was based on the observation that feeding seemed to be necessary before each moulting stage and egg deposition.

In terms of the feeding behaviour of *B. longicaudatus*, Huang & Becker (1997) described four distinct periods: probing, stylet penetration, ingestion, and stylet retraction. The initial contact with the root surface was by rubbing the lip region against the epidermal cells at different sites, even moving from one root to another. Whilst rubbing, the stylet typically probed the root at each site. The nematode needed to arch its anterior body to bring the stylet perpendicular to the root surface. After selecting a suitable feeding site around the root tip region, probing accelerated rapidly with increasing intensity until the stylet tip was inserted into the root. The stylet penetrated slowly as it moved back and forth, with the lips pressed onto the root surface, at which point the valve of the oesophageal median bulb began pulsating. The oesophageal lumen straightened to allow about half of the stylet to penetrate into the root tissue. No body movement was required to aid stylet insertion. The ingestion of plant nutrients began when the valve pulsated more rapidly. During the ingestion period, the nematode's body remained stationary. This could last from 1 minute to 12 hours. When the nematode finished feeding, it withdrew its body to pull the stylet out of the root, and then retracted the stylet into its body. Adequate feeding usually resulted in the nematode's intestine being full of globular material.

2.4.2 Symptoms of damage

In the study by Huang and Becker (1997), the observation was made that brown lesions typically appeared on the roots at the feeding sites, from 12 to 24 hours after the initiation of feeding. Also, the roots sometimes became slightly swollen behind the lesions. When subjected to attack by many nematodes at the same time, the whole root tips turned black and stopped growing. According to Agrios (2012), it is not the direct mechanical injury inflicted by nematodes that causes most of the damage, but the secretion of saliva injected into cells while the nematodes are feeding.

The root damage caused by plant-parasitic nematodes leads to poor root function, in terms of water and nutrient uptake, and root pruning that results in a shallow or stubby root system.

The effects of nematode feeding generally become apparent only when conditions become unfavourable for the growth of turf. The visible above-ground symptoms mimic symptoms of stress e.g. chlorosis, lack of vigour, wilting, and are likely to be most evident under stressful conditions (Smiley *et al*. 2005).

Crow (2015) considers *B. longicaudatus* to be among the most damaging of all plant-parasitic nematodes, with the capacity to kill the root meristem, halt root growth, and attack lateral roots. Affected turf tends to have an abbreviated, stubby-looking root system that appears to be cropped off just below the thatch. Turf damage typically occurs in patches because the nematodes are generally clumped in distribution. Declining turf is prone to infestation with broadleaf weeds such as spurge (*Euphorbia spp.*).

Stirling *et al*. (2013) described the symptoms of *I. lolii* damage to kikuyu. Above-ground symptoms include wilting, yellowing, and poor recovery from damage, leaving an irregular patchwork of bare areas. Root systems are greatly reduced, with short, stubby roots having dark, shrunken lesions, particularly at the tips. Turf replacement on nematode-infested soils results in poor establishment, with severely stunted roots that only grow a few centimetres.

2.4.3 Damage threshold

The University of Florida has produced guidelines with risk thresholds, based on the population densities and species of plant-parasitic nematodes extracted from a soil sample. The risk of turf damage occurring is rated as low, moderate or high. For example, *B. longicaudatus* has a threshold of 25 nematodes per 100 mL of soil, at which the turf is considered to be at high risk of damage. This means that there is likely to be damage to the root system and a decline in turf quality. At a threshold of 10 nematodes per 100 mL of soil, the turf is considered to be at moderate risk, as damage may become evident if the turf is placed under stress conditions (Crow 2014).

There are limitations to the use of guidelines such as those described above. Crow (2014) states that these risk thresholds have been developed for Florida conditions and may not apply in other environments. Also, the nematode numbers given in the guidelines are based on a specific method of extraction from the soil, i.e. sugar floatation with centrifugation, and other methods of extraction may have different efficiencies and give different numbers. The method of sampling is also important because nematodes are not evenly distributed in the soil, and numbers tend to fluctuate depending on the condition of the turf. The risk thresholds are based on measuring the average population density across the area being tested, and are only useful if multiple core samples are combined. There are also many other variable factors related to management practices and environmental conditions that can influence the risk of turf damage.

Stirling *et al*. (2013) stated that data collected from the field over many years have consistently shown problems associated with *I. lolii* on kikuyu at levels as low as 5-10 nematodes per 200 mL of soil. In the absence of a recognised guideline for damage threshold, this finding would suggest that the threshold for *I. lolii* is likely to be similar to *B. longicaudatus*.

2.5 MANAGEMENT

2.5.1 Chemical

According to Crow (2015), *B. longicaudatus* is susceptible to fumigant and nematicide treatments. However, the nematode is difficult to control with chemicals due to its seasonal vertical migration. In turf situations, it will migrate deeper in the soil during the summer months, limiting the effectiveness of treatments applied to the turf surface at that time. The chemicals that were historically most effective against sting nematode had the characteristics of being highly toxic and water soluble or gaseous, in order to move into the soil profile and kill nematodes. These included fumigants, such as methyl bromide, and organophosphate pesticides such as fenamiphos. Over the last 25 years, many of these types of products have been withdrawn by the regulatory authorities for various health and environmental reasons (Crow 2014).

In Australia, there was widespread use of fenamiphos to treat *I. lolii* in turf during the late 1980s (Stirling *et al.* 2013). As it was the only nematicide registered for use on turf at that time, problems developed with enhanced or accelerated biodegradation in some golf and bowling greens where there had been a long history of use. This had the effect of reducing the period that it remained active in the soil. Accelerated microbial degradation of fenamiphos was initially recognised in cropping situations. For example, on tomato-growing soil in Queensland where it was only applied once a year, the nematicidal activity was reduced from the normal six weeks to less than two weeks (Stirling *et al.* 1992). In recognition of this problem, a statement was added to the product label advising users of the potential for reduced effectiveness with repeated use.

New chemical nematicides have been developed and recently introduced to the turf industry in the USA. There are three new active ingredients: abamectin, fluensulfone and fluopyram. Based on extensive turf field data evaluating their efficacy at the University of Florida, these active ingredients are effective on a range of plant-parasitic nematodes, including *B. longicaudatus*. However, each active ingredient has relative strengths and weaknesses. Their efficacy and optimal application timing are influenced by the biology, mode of feeding and seasonal population dynamics of the targeted nematode (Crow 2016a). For example, abamectin is not as effective on *B. longicaudatus*, because the nematicide binds to organic matter in the thatch layer and does not reach the target nematode (Crow 2016b).

In Australia, abamectin and fluopyram have been registered for the control of southern sting nematode in turf, despite limited efficacy data to support this use. Fluensulfone has been trialled but is yet to gain turf registration.

2.5.2 Biological

With the phase-out of fenamiphos, there has been a proliferation of biological alternatives on the market, with many claims that such products are effective against plant-parasitic nematodes. As these products are either exempt from, or have less rigorous pesticide regulatory requirements, there is generally little or no field efficacy data to support such claims. Field experiments conducted by the University of Florida on a range of commercially available biological compounds, found that none of the experimental treatments reduced the populations of plant-parasitic nematodes, including *B. longicaudatus*, or consistently improved turf visual performance or root production (Crow 2005).

A biological treatment that has demonstrated some effectiveness in protecting turf against plant-parasitic nematodes is based on the bacterium *Bacillus firmus* strain I-1582. It is applied to the turf surface and washed into the soil, where it colonises the root system and produces compounds that protect the roots from nematode attack. As the mode of action is root protection rather than killing the nematodes, it is more effective when used to prevent nematode damage, rather than treating an existing problem (Crow 2014). This product is commercially available in the USA, but has not been released in Australia.

Pasteuria is another biological treatment that was developed in the USA for nematode control in turf. This is an endospore-forming bacterium that is capable of parasitising nematodes. *Pasteuria* is host-specific and occurs naturally in the nematode's native habitat. The active ingredient for *B. longicaudatus* is *Candidatus* Pasteuria usgae, which demonstrated good efficacy in growth chamber studies using *in vitro* produced material. However, when it was produced on a commercial scale, with the spores incorporated into a granular clay carrier, it proved to be ineffective in the field (Crow *et al*. 2011).

The *Pasteuria* isolates capable of attacking *B. longicaudatus* have not been tested against *I. lolii*, but may not be effective due to host specificity issues. In an extensive survey by Stirling *et al.* (2013), *Pasteuria* spores were found attached to *I. lolii* at two golf courses in Sydney, indicating that there is potential to find an isolate capable of attacking southern sting nematode.

2.5.3 Cultural

Given that turf damage related to plant-parasitic nematodes generally becomes evident when the turf is under stress, management practices that reduce turf stress will help to counteract the negative effects of the nematodes. For example, turf that has extensive root damage requires more frequent, light watering to keep it from wilting. Similarly, damaged roots have a reduced capability to take up nutrients, requiring more frequent, light fertilising to avoid nutrient deficiency (Crow 2014). These are general principles based on field observations and experience. No research was found into the effects of watering and fertilising practices for nematode management, except in conjunction with nematicide applications. Trenholm *et al.* (2005) demonstrated that chemical control of *B. longicaudatus* alleviated drought stress in turf under limited irrigation. Luc *et al*. (2007) found that fertilising with nitrogen improved turf performance following the application of a nematicide.

Crow (2014) also commented that some organic soil amendments, such as composted manures, may reduce nematode damage and assist turf recovery after damage has occurred. The beneficial effects of organic amendments have been studied extensively and are worthy of further attention. There are various mechanisms involved, including the release of toxic compounds from decomposing materials, stimulation of antagonistic microorganisms, modification of the nematodes' habitat, and improved plant health leading to better tolerance of plant-parasitic nematodes (McSorley 2011).

Rodriguez-Kabana *et al.* (1987) state that the most efficacious organic materials against plant-parasitic nematodes are those with low carbon to nitrogen ratios (C:N) that release ammonia (NH_3) into the soil. Organic materials with C:N in the range of 15-20 are considered optimal to give nematicidal activity without phytotoxicity; C:N >20 usually does not have nematicidal activity, while C:N <10 can cause phytotoxicity. A limitation on the use of ammonia-releasing organic amendments is that the high application rates required for satisfactory control have the potential to damage turf grass. Therefore, the focus should be on improving plant nutrition, increasing the soil's water-holding capacity and encouraging natural enemies to enhance biological suppression of plant-parasitic nematodes.

2.6 FUTURE RESEARCH AND EXTENSION NEEDS

Future research and extension programs in turf grass should aim to minimise the spread of *I. lolii* within Australia and provide more effective strategies for managing the nematode. Given the limited study of *I. lolii*, the knowledge gained from extensive research of *B. longicaudatus* in the USA provides very useful guidance.

2.6.1 Biosecurity

Since its introduction, *I. lolii* has become widespread in turf grass within certain coastal regions of New South Wales and Western Australia. However, there are still large areas of Australia that are not infested. While there is an obvious need to limit the spread of this nematode, Stirling *et al*. (2013) commented that state biosecurity authorities and the turf industry had failed to take action. Unfortunately, this situation has not changed. One of the obstacles is the diverse nature of the turf industry e.g. turf farms, golf courses, bowling greens, sports fields, parks, school grounds, racecourses, tennis courts, and commercial and domestic lawns. This means that there is no single representative organisation to deal with biosecurity authorities. Another problem is the high cost of implementing a suitable biosecurity scheme and how it is funded.

To ascertain the extent of *I. lolii* distribution, independent nematode surveys are required within the various segments of the turf industry. From this information, relevant advice can be given to practitioners on how to minimise its spread. Infested turf farms are a major threat, because of their potential to distribute the nematode widely and rapidly. An accreditation procedure for turf farms involving regular nematode testing is required. However, such a scheme would need the support of state regulatory authorities, due to the reluctance of the commercial turf production sector to self-regulate.

One of the challenges in detecting sting nematode infestations on turf farms is the intensity of sampling that is required on vast production areas, making the process quite expensive. Southey (1986) described how the probability that a particular nematode will be detected in a representative soil sample depends on the average population density in the area sampled. For example, the field average would need to be 10 million nematodes/ha in the top 10 cm, or 1 nematode/100 mL of soil, to give a 99% chance of detection in a sample examined in the laboratory. The chance of detection drops to 39% for an average population of 1 million nematodes/ha, or 1 nematode/L of soil. This illustrates the difficulty of developing a protocol to detect a low population of sting nematode on a turf production area.

The threat of *I. lolii* to agricultural production in Australia also needs to be taken seriously. Stirling *et al*. (2013) raised the concern that kikuyu and ryegrass are widely used pasture grasses in the grazing industry. Their study also demonstrated that sugarcane is an excellent host. Consequently, they concluded that host-range studies are required on other grass crops, such as cereals and maize, as these may be at risk from introduction of the nematode.

2.6.2 Nematode diagnostic services

Nematode analysis has an important role in identifying and monitoring infestations of *I. lolii* in turf. The Australian turf industry currently has limited options for laboratories with suitable expertise to provide analytical services for plant-parasitic nematodes. These laboratories use the Whitehead-tray technique for nematode extraction (Whitehead & Hemming 1965) and microscopic examination. A disadvantage of this method is that the extraction efficiency can be reduced by mishandling of the sample, such as overheating in transit to the laboratory or excessive mixing of the soil.

To improve the accuracy and reliability of nematode analysis, there is a need to develop cost-effective molecular testing for soil samples from turf. This would be equivalent to the service provided by the South Australian Research and Development Institute (SARDI) for agricultural crops (Ophel-Keller *et al.* 2008). The economic viability of this type of service should be investigated, with the initial focus being the detection and quantification of *I. lolii*.

Sampling methodology is another critical aspect of testing for *I. lolii* in turf. This relies on an experienced agronomist/consultant with the ability to spot the symptoms of turf damage in the field, using an appropriate sampling method for the situation and then interpreting the results for the turf manager.

The development of damage thresholds for *I. lolii* in turf would seem to have limited practical application, given the variable factors that influence the turf's ability to cope with nematode infestation. Any thresholds that were developed through experimental work, either in pots or field trials, would be theoretical in nature. Given the pathogenicity of *I. lolii* in turf, there is probably little to be gained by establishing a guideline for an acceptable population density in the soil.

2.6.3 Population dynamics

An understanding of the population dynamics of *I. lolii* in turf is essential for developing better management strategies. The study of *B. longicaudatus* in southern California by Bekal & Becker (2000a) found that there were seasonal fluctuations in nematode population and vertical migration within the soil profile. Identifying the periods of highest population pressure and vertical movement of *I. lolii* should be of great interest to Australian turf managers. This knowledge would influence the type of control measures used and the timing of treatments to improve their effectiveness.

2.6.4 Management of turf stress

As turf infested with *I. lolii* is far more susceptible to stress from having a shallow and dysfunctional root system, maintenance inputs such as water and nutrients need to be increased accordingly. The ramifications include higher maintenance costs, increased demand on limited water supplies, and the potential environmental impact from nutrient leaching. Therefore, research is needed to quantify the water and nutrients requirements of *I. lolii*-infested turf, with the aim of developing best-practice guidelines for irrigation and fertilising.

2.6.5 Resistant/tolerant turf

The evaluation of turf species and cultivars with improved resistance and tolerance to *I. lolii* should be a high priority for research in Australia, as it offers a sustainable management strategy for this nematode. The turf species that are applicable to sports turf situations are couch, kikuyu, ryegrass and bentgrass, each having a range of cultivars available for evaluation. With kikuyu being particularly susceptible to damage (Stirling *et al*. 2013), couch is of most interest as an alternative warm-season grass species. Examples of couch cultivars that are locally available include TifTuf, Grand Prix and the industry standard Wintergreen. Another cultivar that warrants evaluation is TifSport hybrid couch, which showed promise against *B. longicaudatus* in a study by Pang *et al.* (2011). For non-sports turf situations, other species that could be considered include seashore paspalum and Queensland blue (*Digitaria didactyla*), based on the author's observations of them growing reasonably well in parks infested with *I. lolii*.

2.6.6 Organic amendments

The application of organic soil amendments has great potential for managing *I. lolii* in turf, especially using composted blends of manure and green waste. In the Perth region, there has been extensive use of composted materials on *I. lolii* infestations, either incorporated before planting or top dressed on established turf. Beneficial effects have been observed with high rates (i.e. 100-250 m^3/ha), such as nematode suppression, improved root growth and better surface stability. The disadvantages are the high cost, the potential for organic matter to impede drainage, and excessive nitrogen and phosphorus loading (K. Johnston & P. Ruscoe, unpublished).

Further research into locally available sources of recycled organic waste material for nematode management is warranted in the turf industry. The key questions to address are whether economically feasible application rates will enable turf grasses to better tolerate the effects of southern sting nematode, through improved soil health and plant growth; and whether biological suppressiveness will be enhanced to the point that nematode populations are reduced.

2.6.7 Biological treatments

There are currently no effective biological treatments available for plant-parasitic nematodes on turf in Australia. Further investigation is needed to find an *I. lolii*-specific isolate of *Pasteuria* that could be developed as a biological nematicide, as has been done in Florida for *B. longicaudatus*. Another bacterial product, *Bacillus firmus*, should be tested on turf infested with *I. lolii* to assess whether it provides any root protection from nematode feeding. A great deal of development work would be required to prove the effectiveness and then commercialise these types of products.

2.6.8 Nematicides

In the last few years, various manufacturing companies have funded extensive trial work to evaluate nematicides on turf in Australia. Given the slow progress with registration of these products, it can be assumed that similar problems with treatment efficacy have been encountered to those reported by Crow (2016) in the USA. However, evaluation of chemical nematicides should continue as new products are constantly being developed. The most recent example is fluazaindolizine, which is reported to be highly effective and selective for the control of plant-parasitic nematodes, with a unique mode of action (Lahm *et al.* 2017).

2.6.9 Extension

With the diversity of the turf industry in Australia, it is a major challenge to disseminate research findings and technical information to the various segments. As there are no extension services provided by government or universities, and very few independent consultants, most turf managers tend to rely on company sales representatives to provide information and advice. The best mechanism for reaching turf managers is through the various turf industry associations, via their publications, E-newsletters and events. Thus, it is important that biosecurity authorities and researchers working with *I. lolii* utilise these means to continually pass on new information to those who are responsible for the production, installation and management of turf grasses.

Chapter 3. Research: Survey of southern sting nematode and other plant-parasitic nematodes on municipal sports fields in the Perth region of Western Australia.

3.1 INTRODUCTION

The southern sting nematode (*Ibipora lolii*) is a serious problem on turf grass in Australia, capable of causing severe damage from feeding on the root system. Symptoms include shallow and dysfunctional roots, increased susceptibility to wilting and nutrient deficiency, and the development of bare patches that often require turf replacement. Once the nematode has been introduced to a site, the infestation is likely to remain there indefinitely. Some sites are suspected to have been infested since the 1970s (Stirling *et al*. 2013).

I. lolii was originally identified by Siviour & McLeod (1979) from couch bowling greens and bentgrass golf greens in the Newcastle area of New South Wales. Based on diagnostic samples from turf grass in New South Wales, *I. lolii* was most prevalent in the mid-north coastal region between Gosford and Taree, a distance of ≈200 km, centred on Newcastle. It was also detected further north in Coffs Harbour, and in the south-eastern coastal suburbs of Sydney (Stirling *et al.* 2013).

Morulaimus gigas was first described in Western Australia by Nobbs & Eyres (1992), from a couch bowling green in Dandaragan, ≈170 km north of Perth. Nambiar *et al.* (2010) summarised the results of diagnostic samples collected from turf grass in all Australian states, reporting new detections of *M. gigas* in Western Australia, South Australia, Victoria, New South Wales and Queensland. The nematode described as *M. gigas* was later found to be identical to *I. lolii* (Stirling *et al*. 2013).

Results of diagnostic samples from turf grass in Western Australia showed that *I. lolii* occurred in more than 40% of the samples (Stirling *et al.* 2013). Most of the sites tested were within 50 km of Perth, with a high proportion (≈80%) of samples from sports fields or recreational areas where kikuyu was the dominant turf species.

The Perth region of Western Australia is located on a land formation known as the Swan Coastal Plain, a narrow strip of land (15-30 km wide) between the Darling Range and Indian Ocean. It is predominantly composed of a series of coastal sand dune systems, shown in

Figure 3.1 as the grey, orange and yellow shaded areas. The sand content (particle size 0.05-2.0 mm) is typically >95%, and silt/clay (<0.05 mm) is <5%. The sandy soils of the Swan Coastal Plain provide an ideal habitat for the southern sting nematode on irrigated areas of turf grass.

It is estimated that there are approximately 500 sports fields managed by local government authorities across the Perth region, covering an area of more than 1000 hectares. These are used for community sporting competitions, including Australian rules football (AFL), soccer, rugby, hockey, lacrosse, cricket, baseball and softball. There are 30 municipal councils within the Perth region, the boundaries of which are marked on Fig. 3.1. Southern sting nematode is a major concern on kikuyu sports fields, because playing surfaces are prone to becoming badly degraded and unsafe. Infested grounds have significantly higher maintenance costs, including the need for more turf replacement.

Previous surveys of diagnostic samples may not represent the actual distribution of *I. lolii*, given the potential bias towards sites having nematode problems. A systematic, randomised survey was needed to provide an accurate indication of nematode incidence on municipal sports fields in the Perth region. The aims of this survey were to ascertain the distribution of *I. lolii*, identify other plant-parasitic nematodes, and make general observations of the turf, including species and symptoms of nematode-related damage.

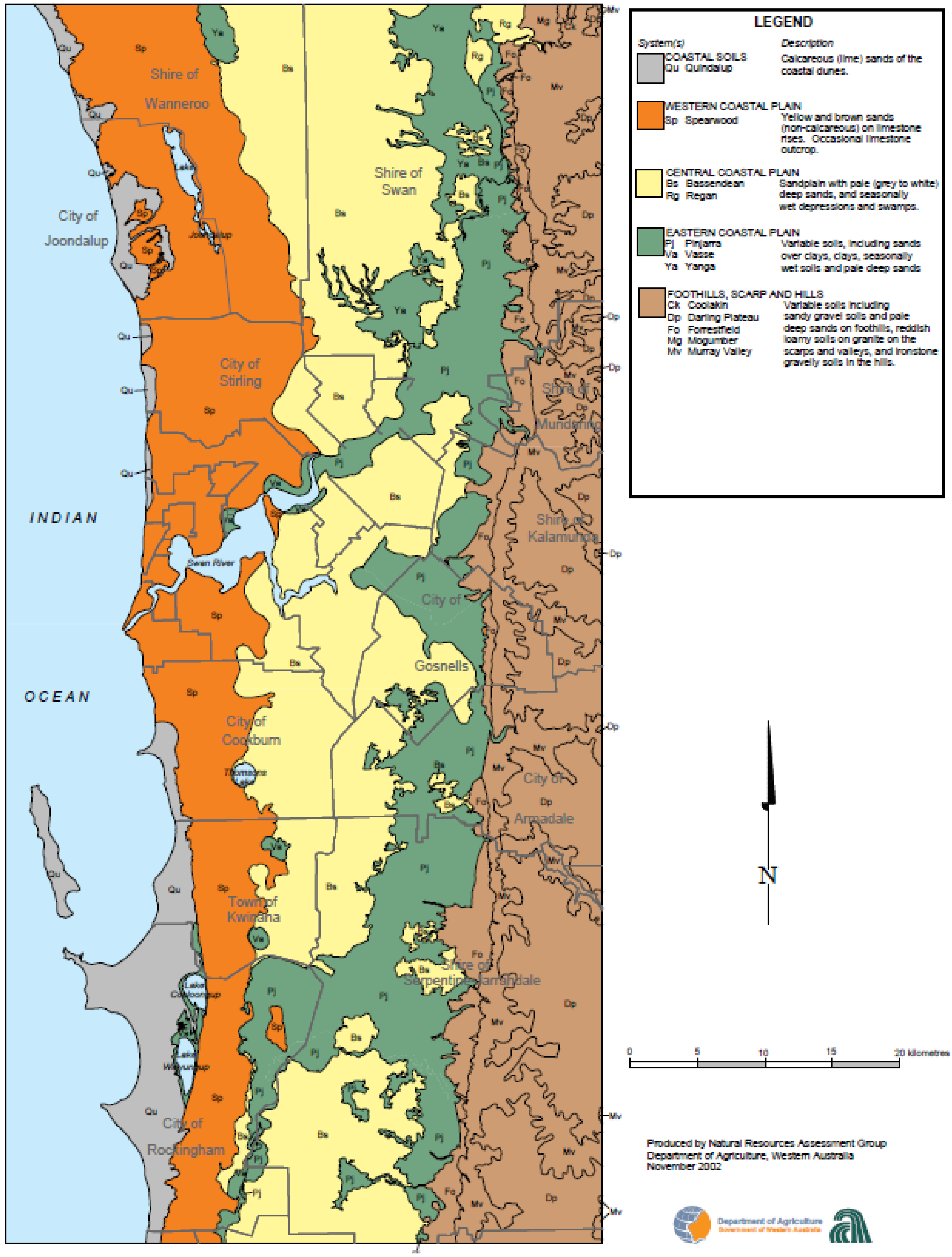

Figure 3.1. Soil-landscape systems of the Perth urban area showing local government boundaries (Department of Agriculture WA, 2002).

3.2 METHODOLOGY

A total of 90 sports fields were sampled in 21 municipal council areas across the Perth region, over a 4-month period from May to September 2015. The sizes of the sports fields varied, but were typically about 1 ha for soccer or rugby, and up to 2 ha for AFL or cricket. In each of the council areas that were surveyed, approximately 20% of the sports fields were randomly selected from a list of sites, using a random number generator (Excel RANDBETWEEN function).

At each survey site, an overall representative soil sample was collected by taking 15 cores/ha in a grid pattern, using a 20 mm-diameter core sampling tool to a depth of 10 cm. Whilst sampling, observations were made of the turf species and symptoms of nematode-related damage e.g. sparse turf cover, shallow root system and weed infestation. The soil cores were mixed gently and thoroughly to form a composite sample for nematode analysis. Nematodes were extracted from a 200 mL sub-sample of soil placed on a tray and were retrieved after 2 days using a 38 µm sieve (Whitehead & Hemming 1965). A compound microscope was used at 40X magnification to identify *I. lolii* and quantify the population density. Other plant-parasitic nematodes were identified to generic level and also counted.

Kikuyu *(Pennisetum clandestinum)* was the most common species of turf grass on the survey sites. Other turf species were generally present in a mixed sward with kikuyu, including couch *(Cynodon dactylon)*, seashore paspalum *(Paspalum vaginatum)*, Queensland blue *(Digitaria didactyla)*, ryegrass *(Lolium perenne)* and bentgrass *(Agrostis stolonifera)*.

Based on observations of nematode-affected turf on the survey sites, further sampling was undertaken to better understand the distribution of *I. lolli* within the sports fields. Ten sites from different council areas where the nematode was detected in the survey were chosen for sampling, between January and April 2016. Within each site, three representative locations were selected: a good area with no symptoms of damage; a moderately affected area with slight symptoms; and a poor area with severe symptoms. Soil samples consisting of 10 cores, 20 mm in diameter and 10 cm deep, were taken from each location for nematode extraction to compare the numbers of *I. lolii*. The turf root system was assessed at each location by taking five core samples, 50 mm in diameter, and recording the average depth of the longest root in each core.

3.3 RESULTS

There were nine different types of plant-parasitic nematodes found in the survey, as listed in Table 3.1. Southern sting was detected in approximately half of the survey sites, although the mean population density across all of the sites where it was detected was relatively low. Other nematodes that were more prevalent included dagger, stubby-root, sheath and spiral, with sheath being the most commonly occurring. *Sphaeronema* had by far the highest mean number and individual count, followed by dagger. Stubby-root nematode was quite widespread, but had a very low mean population density.

Table 3.1. Occurrence and population densities of plant parasitic nematodes (averaged across the sites in which the nematodes were detected, shown as mean ± standard error) on 90 randomly-selected sports fields in the Perth region of Western Australia.

Common Name	Genus/species	Occurrence (% of sites)	No. nematodes/200 mL soil	
			Mean ± SEM	Highest count
Southern sting	*Ibipora lolii*	51	27 ± 4	96
Dagger	*Xiphinema*	77	120 ± 16	684
Stubby-root	*Paratrichodorus*	82	12 ± 1	76
Sheath	*Hemicycliophora*	89	55 ± 7	380
Spiral	*Helicotylenchus etc.*	68	27 ± 4	156
Root-knot	*Meloidogyne*	51	7 ± 1	34
Lesion	*Pratylenchus*	23	15 ± 8	168
Ring	*Criconemella etc.*	14	3 ± 1	16
	Sphaeronema	48	503 ± 122	3670

On most of the survey sites, infestations of *I. lolii* were associated with obvious symptoms in the turf, including shallow root systems, sparse turf cover, weeds, potholes and bare patches. In contrast, these symptoms were not consistently observed with the other types of plant-parasitic nematodes, even at very high population densities. Turf species other than kikuyu, especially couch, often occurred in the sites infested with *I. lolii*, possibly indicating better tolerance to the nematode.

It was also observed in the survey that infestations of *I. lolii* were not evenly distributed within the sports fields. This was supported by the findings of further sampling on ten of the survey sites (Table 3.2). The symptoms of turf damage, especially root depth, were a good indicator of the variation in nematode population density.

Table 3.2. Population densities of *I. lolii* (averaged across all sites, shown as mean ± standard error) and turf root depth measured in core samples taken from representative locations on ten sports fields selected from the nematode survey.

Representative location	Mean no. *I. lolii/* 200 mL soil ± SEM	Mean root depth (mm) ± SEM
Good area: no damage	26 ± 24	156 ± 6
Moderate area: slight damage	131 ± 28	116 ± 6
Poor area: severe damage	202 ± 34	77 ± 4

All of the poor areas of turf were infested with *I. lolii*, and it was present in eight out of the ten moderate areas. In the good areas of turf, the nematode was not detected in seven sites. The mean number for the good areas was skewed by one site that contained a very high population of *I. lolii* (237/200 mL soil) with no obvious symptoms in the turf. This location had favourable conditions for the turf, such as low traffic, high organic matter content in the soil and high moisture content.

3.4 DISCUSSION

The survey results indicate that southern sting nematode is widespread on municipal sports fields across the Perth region. Yet its absence from almost half of the survey sites highlights an opportunity to prevent further spread with appropriate biosecurity measures. The most common method of introduction to a new site is on infested planting material e.g. turf rolls. As some turf farms in the Perth region are known to be infested, it is critical for turf managers to ensure that planting material is sourced from non-infested production areas.

On the survey sites, the infestations of southern sting nematode were generally localised in high-traffic areas, e.g. within the centre corridor or under floodlights, presumably where turf had been worn out and replaced in the past. By taking overall representative samples, rather than targeting the worst areas of turf, the nematode numbers generally reflected the extent of infestation within each sports field. For example, a low number (<10/200 mL) indicated that the infestation was confined to small, localised areas; whereas a high number (>50/200 mL) indicated that the infestation was spread over a large area of the sports field.

The follow-up sampling of localised infested areas with symptoms of turf damage, found that the population densities of southern sting nematode were much higher than in the survey samples from the same sites. Therefore, the method of sampling has to be considered when interpreting nematode counts in terms of the damage threshold or level of risk to the turf (Crow 2014).

The other plant-parasitic nematodes that were found in the survey appeared to have relatively low pathogenicity on turf grasses in sports fields. This was based on observations of the good condition of turf where there were high population densities of nematodes other than southern sting. *Sphaeronema* does not appear to be pathogenic to turf grass, even at very high population densities.

The species have not been confirmed for the plant-parasitic nematodes found in this survey, other than southern sting. There is a need to identify these nematodes to species level, using a combination of taxonomic assessment and DNA sequencing. Also, plant-parasitic nematodes other than those detected in this survey may occur on turf grass in the Perth region. An example is cyst nematode (*Heterodera*), although it would be of very minor importance.

Chapter 4. Research: Population dynamics and depth distribution of southern sting nematode in kikuyu turf.

4.1 INTRODUCTION

The southern sting nematode (*Ibipora lolii*) is a destructive parasite of turf grass that is most likely to have been introduced to Australia from Central or South America, where other species of *Ibipora* are found. It is a relatively large nematode (≈2.5 mm long) that thrives in sandy soils, and is usually associated with very poor root growth and patchy appearance of turf grass. *I. lolii* is most prevalent in two distinct coastal regions on opposite sides of the country: along the mid-north coast of New South Wales, centred around Newcastle; and the Perth metropolitan area of Western Australia. There is strong evidence to indicate that the nematode was transferred from Newcastle to Perth on infested couch (*Cynodon dactylon*) planting material for bowling greens. It has since been spread to many sporting fields around the Perth region, where kikuyu (*Pennisetum clandestinum*) is the dominant turf species (Stirling *et al*. 2013).

A study of the sting nematode (*Belonolaimus longicaudatus*) on golf course fairways in the inland desert region of California, USA found that its population dynamics were generally correlated to soil temperature. On couch/bermudagrass (*C. dactylon*) that was overseeded with perennial ryegrass (*Lolium perenne*), significant population increases did not occur until the soil temperature reached 20°C, and the numbers peaked in the summer or autumn months (Bekal & Becker 2000a).

There is a need to understand the seasonal population dynamics of *I. lolii* and the influence of environmental factors, such as soil moisture and temperature. The climatic conditions of the Perth region in Western Australia are characterised by strongly seasonal rainfall and temperature patterns. The long-term mean annual rainfall is 767 mm, almost 80% of which occurs in the winter months of May to September. During summer, conditions are hot and dry, with mean monthly maximum temperatures ranging from 29 to 32°C. Irrigation is required on turf grass from October to April, as the mean daily pan-evaporation ranges from 5 to 10 mm. The typical water requirement to maintain kikuyu in suitable condition is 3 to 6 mm per day.

This study focussed on *I. lolii* in the Perth region with the aim of identifying any key periods when nematode multiplication occurred, and ascertaining whether there was any vertical movement of the nematode within the soil profile.

4.2 METHODOLOGY

Population densities of *I. lolii* were monitored at two municipal sports fields in the Perth region for 2 years, from April 2016 to April 2018. The monitoring sites were at Lightning Park in Noranda (Lat. -31.87, Long. 115.91), which is 15 km inland, and Charles Riley Reserve in North Beach (Lat. -31.87, Long. 115.76), 14 km to the west and near the coast. Both sporting fields have deep sandy soil profiles (>95% sand, 0.05-2.0 mm particle size) and had southern sting nematode introduced on kikuyu planting material. Lightning Park was established in 2007 and had very high population pressure of *I. lolii* (>250/200 mL soil), with symptoms of patchy turf cover and shallow root systems. Charles Riley was established in 1999 and had lower population pressure (<100/200 mL soil) during the monitoring period with less severe symptoms in the turf. Both sites were irrigated from October to April, with annual water usage of approximately 1000 mm (10 ML/ha).

At both monitoring sites, three plots (2 x 2 m) were permanently marked in close proximity (10-20 m apart) to ensure that soil samples were taken from exactly the same locations. These plots were situated in the surrounds of the playing fields, where no treatments were applied that could have potentially interfered with nematode population dynamics. Each plot was sampled separately every two months at a depth of 0-10 cm, and twice per year (summer and winter) at 10-30 cm, 30-50 cm, and 50-70 cm. The samples consisted of 9 cores/plot for 0-10 cm, and 2 cores/plot for the other depths, taken using a 20 mm-diameter core sampling tool. For deeper sampling, the top 10 cm was removed using a 50 mm-diameter core sampling tool, and care was taken to avoid mixing soil from different depths.

Nematodes were extracted from a 200 mL sub-sample of soil placed on a tray and were retrieved after 2 days using a 38 µm sieve (Whitehead & Hemming 1965). *I. lolii* was counted as male, female and three juvenile stages. Juveniles were classified according to their size ranges in three moulting phases: J2<625 µm; J3 625-1250 µm; J4 >1250 µm. The nematode length was measured using a scale bar in the eyepiece of a compound microscope at 40X magnification. Nematodes were counted in each plot, with the mean numbers reported for each sampling date.

Soil moisture content and temperature data were available at Lightning Park, from continuous monitoring equipment located in the playing field adjacent to the monitoring site. This consisted of an *Enviro-pro* 40 cm sensor and *Outpost Central* data logger owned by the council to support their irrigation management.

4.3 RESULTS

Southern sting nematode exhibited a pattern of seasonal fluctuation in population density at both of the monitoring sites, although at different population densities (Fig. 4.1 & 4.2). The mean number of *I. lolii*/200 mL soil ranged from 248 ± 57 to 677 ± 103 at Lightning Park, compared with 21 ± 9 to 92 ± 53 at Charles Riley Reserve. The population density peaked during October in both years at Lightning Park and in the first year at Charles Riley Reserve, while the lowest number occurred in April and December at the respective sites.

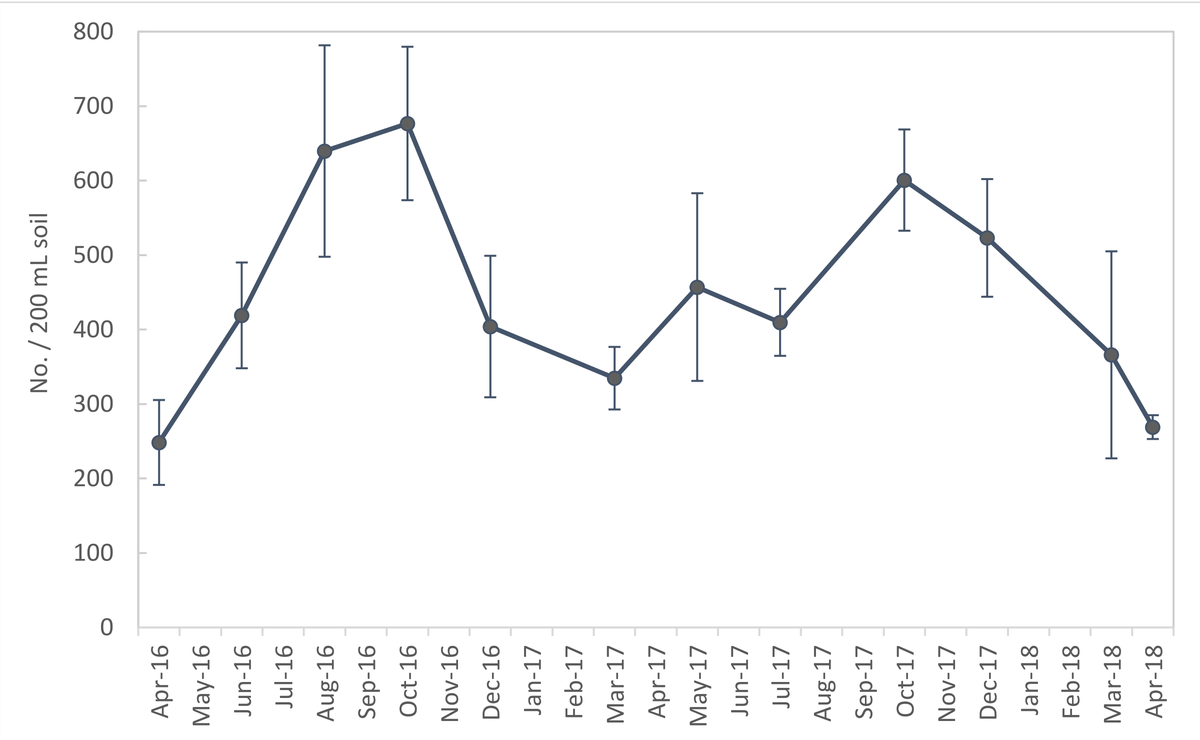

Figure 4.1. Population density of *Ibipora lolii* (0-10 cm) at Lightning Park over a 2-year period. Values are the means (n=3) of all nematode life-stages, with error bars representing the standard error of each mean.

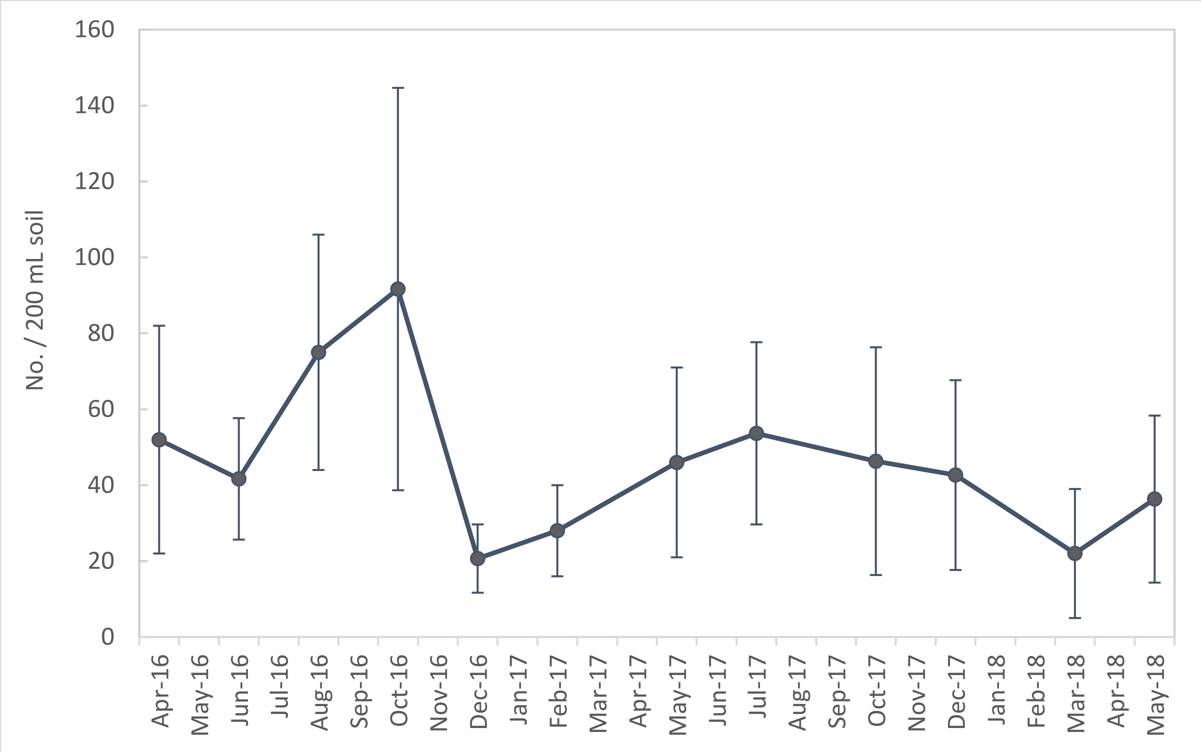

Figure 4.2. Population density of *Ibipora lolii* (0-10 cm) at Charles Riley Reserve over a 2-year period. Values are the means (n=3) of all nematode life-stages, with error bars representing the standard error of each mean.

At most sampling dates, there was a large standard error of the mean population, indicating the extent of variation between plots. For example, the individual plot counts varied from 357 to 812 *I. lolii*/200 mL at Lightning Park in August 2016, and 35 to 197 *I. lolii*/200 mL at Charles Riley Reserve in October 2016. This inter-plot variation demonstrated the uneven distribution of the nematode within these sites.

The mean monthly soil temperature at Lightning Park (Fig. 4.3) was 12-16°C at 10 cm between April and October, when the increase in nematode population occurred. From November to March, when the population declined, it was 20-23°C. The soil temperature range during the monitoring period was a minimum of 8.0°C to a maximum of 30.4°C. The soil moisture content at Lightning Park (Fig. 4.4) was generally at consistently high levels during the winter rainfall period from May to September. In November 2016, there was a severe drying event due to an irrigation system breakdown. The moisture content in top 10 cm did not recover during the summer, although it was evident that irrigation reached the lower depths (10 to 40 cm). In the following summer, a higher moisture level was maintained at 10 cm, although there was drying between irrigation events.

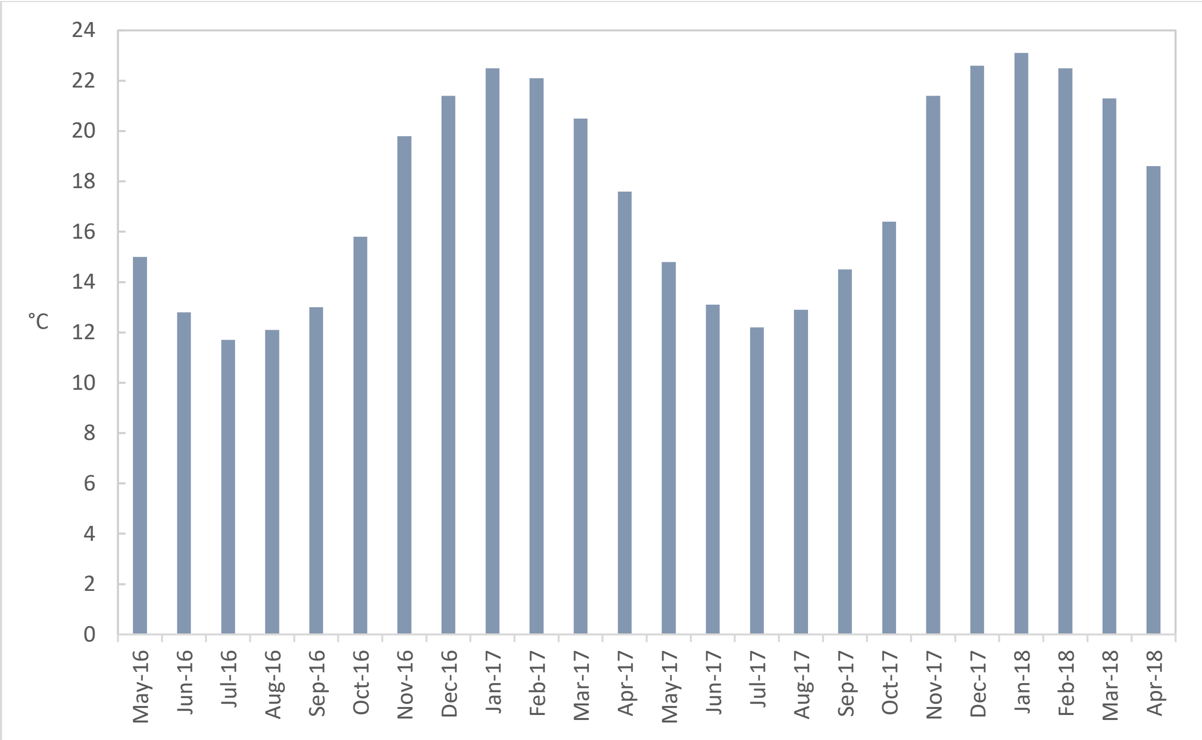

Figure 4.3. Mean monthly soil temperature (10 cm) at Lightning Park, based on hourly measurements.

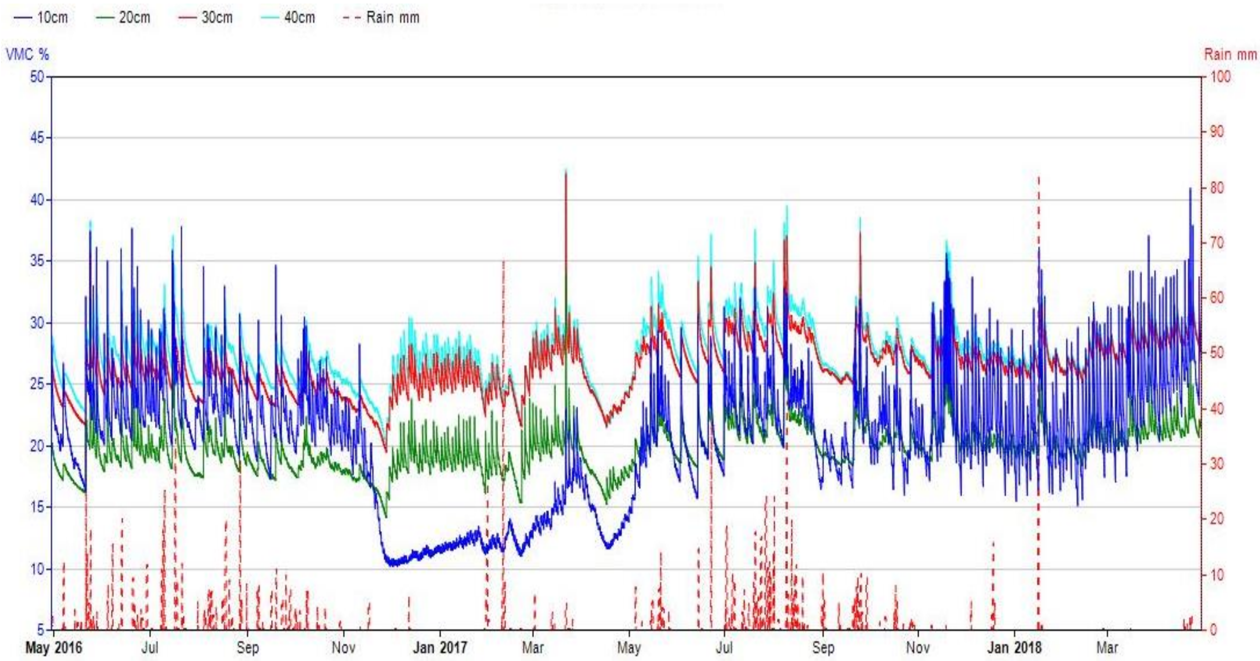

Figure 4.4. Soil volumetric moisture content at depths of 10 cm, 20 cm, 30 cm and 40 cm at Lightning Park, and rainfall recorded in Perth by the Bureau of Meteorology.

Juvenile stages of *I. lolii* were present at every sampling date, indicating that the nematode reproduces all-year-round. Figure 4.5 shows that a distinct peak in the number of juveniles occurred during the month of October in both years of monitoring. The breakdown of juveniles shown in Figure 4.6, indicates that the increase in population was predominantly in the J2 and J3 stages. Fluctuations in the juvenile population tended to reflect the seasonal pattern of the total population. In the adult stage, the numbers of males and females were similar throughout the monitoring period, as shown in Figure 4.7, with neither clearly dominating.

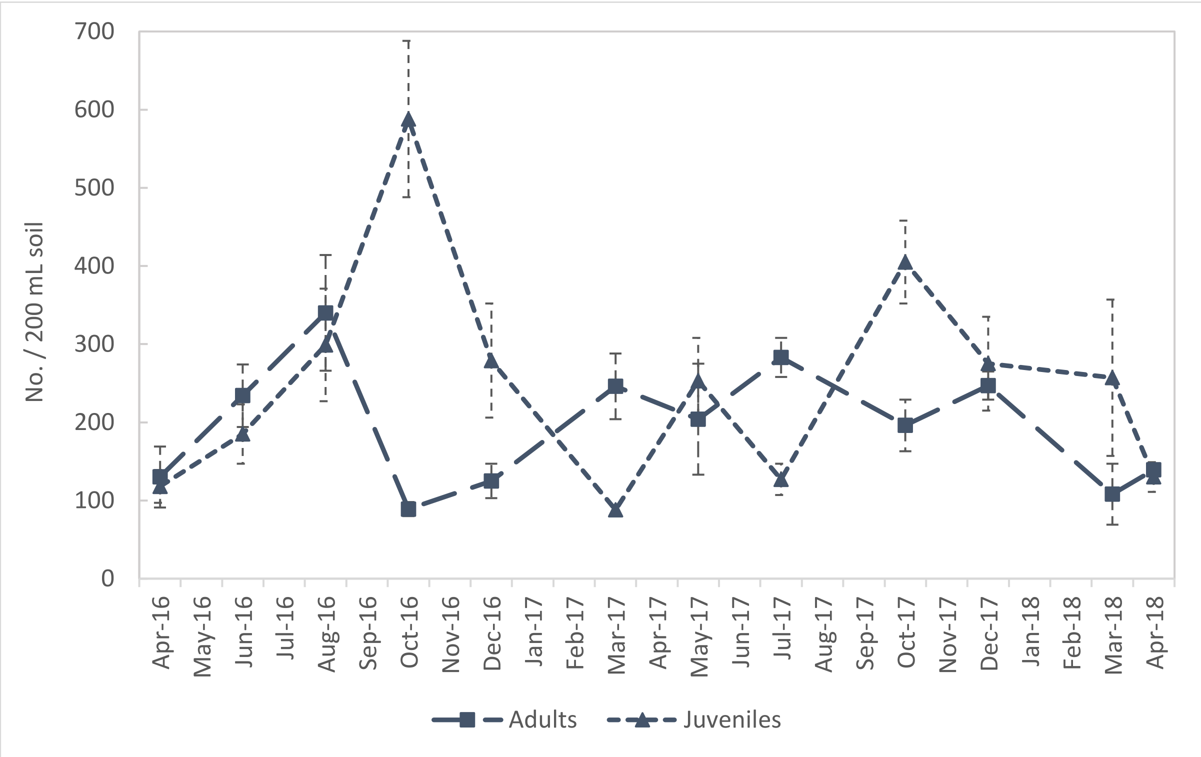

Figure 4.5. Population densities of *Ibipora lolii* adults and juveniles (0-10 cm) at Lightning Park over a 2-year period. Values are the means (n=3) of adults (males + females) and juveniles (J2+ J3+J4), with error bars representing the standard error of each mean.

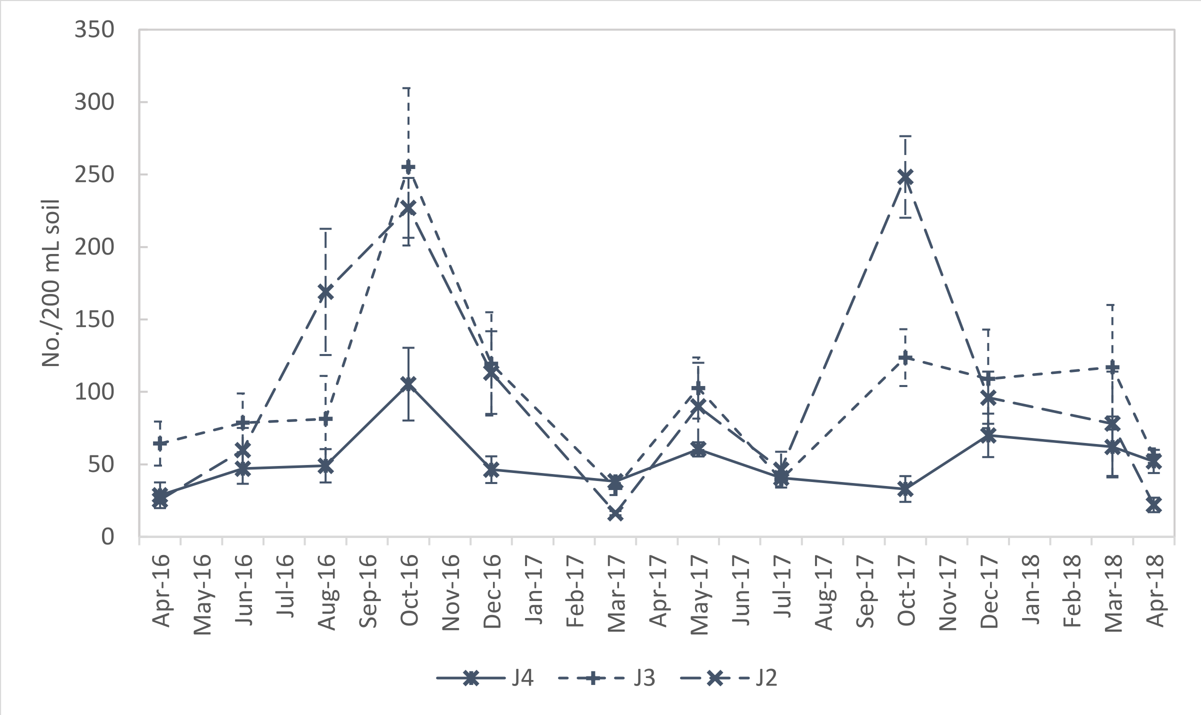

Figure 4.6. Population densities of *Ibipora lolii* juvenile stages (0-10 cm) at Lightning Park over a 2-year period. Values are the means (n=3) of J2, J3 and J4, with error bars representing the standard error of each mean.

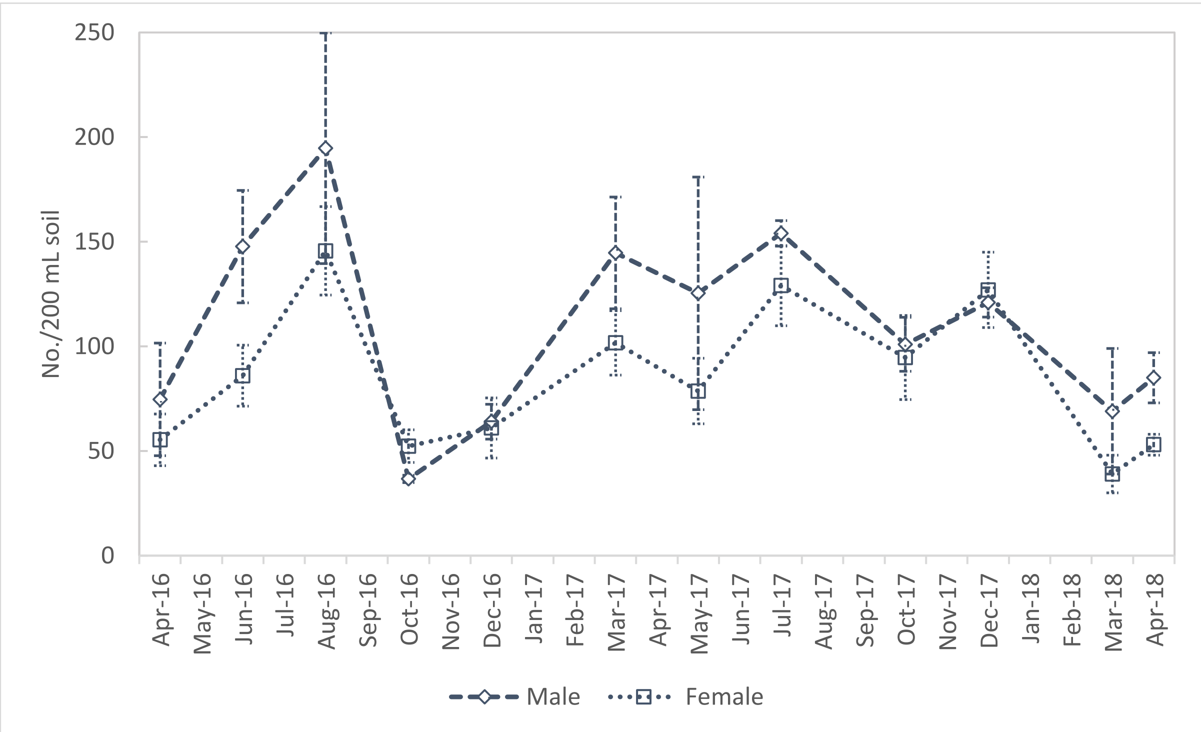

Figure 4.7. Population densities of *I. lolii* males and females (0-10 cm) at Lightning Park over a 2-year period. Values are the means (n=3) of males and females, with error bars representing the standard error of each mean.

In terms of the vertical distribution of *I. lolii* in the soil profile, the highest population density was in the top 10 cm. The nematode was also found at all sampling depths to 70 cm, demonstrating that it is capable of deep vertical movement. Tables 4.1 and 4.2 show that this was the case at both monitoring sites, albeit at different population densities. Throughout the monitoring period, there were males, females and three juvenile stages found at all sampling depths from 10 to 70 cm, as depicted in Figure 4.8.

Table 4.1. Population densities of *Ibipora lolii* at depths of 0-10, 10-30, 30-50 and 50-70 cm at Lightning Park. Values are the means (n=3) and standard errors of all nematode life-stages.

Depth	Mean no. nematodes/200 mL soil ± SEM			
	June 2016	February 2017	July 2017	March 2018
0-10 cm	419 ± 71	335 ± 42	410 ± 45	366 ± 139
10-30 cm	61 ± 17	26 ± 6	39 ± 14	110 ± 43
30-50 cm	51 ± 9	26 ± 5	24 ± 3	45 ± 9
50-70 cm	77 ± 35	50 ± 10	24 ± 5	45 ± 20
Total	607 ± 105	437 ± 46	497 ± 60	565 ± 164

Table 4.2. Population densities of *Ibipora lolii* at depths of 0-10, 10-30, 30-50 and 50-70 cm at Charles Riley Reserve. Values are the means (n=3) and standard errors of all nematode life-stages.

Depth	Mean no. nematodes/200 mL soil ± SEM			
	June 2016	February 2017	July 2017	March 2018
0-10 cm	42 ± 16	28 ± 12	54 ± 24	22 ± 17
10-30 cm	16 ± 6	8 ± 4	8 ± 4	5 ± 3
30-50 cm	16 ± 5	8 ± 2	10 ± 5	9 ± 5
50-70 cm	32 ± 10	11 ± 6	9 ± 4	16 ± 8
Total	106 ± 34	56 ± 22	81 ± 33	52 ± 26

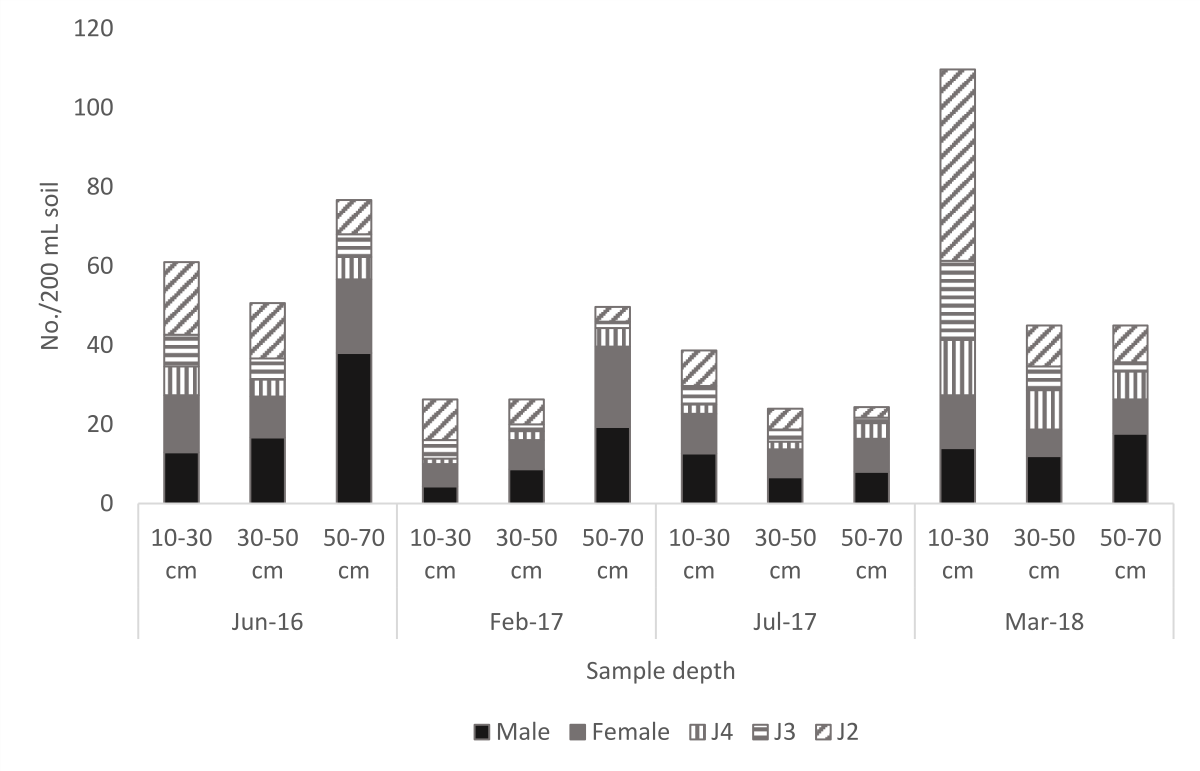

Figure 4.8. Population densities of *Ibipora lolii* males, females and juvenile stages at depths of 10-30, 30-50 and 50-70 cm at Lightning Park. Values are the means (n = 3) at each depth.

4.4 DISCUSSION

Based on the findings of this study, the cool and wet conditions in the winter rainfall period, from May to September, were more favourable for southern sting nematode reproduction than the hot and dry period of the irrigation season, between October and April.

This was an unexpected finding given that in California, Bekal & Becker (2000a) reported that the highest population densities of sting nematode (*B. longicaudatus*) occurred in warm soil conditions (>20°C) during the summer or autumn months. In contrast, the increase in population of southern sting nematode occurred while the soil temperature was below 20°C. However, the California study was on golf courses with daily irrigation, where soil moisture was presumably not a limiting factor.

Bekal & Becker (2000a) also found that peaks in population density were followed by rapid declines, probably because the food supply became limited from severe damage to the root system by the nematode. In contrast, the decline in population of southern sting nematode seemed to be related more to seasonal environmental factors, such as higher temperature and depletion of soil moisture in the top 10 cm. In the Perth region, the highest growth rate of kikuyu occurs from late summer through to autumn (Barton *et al*. 2009). This was the period when the nematode population was generally found to be at its lowest.

The distinct peak in juvenile population of southern sting nematode during the month of October may be an important finding in terms of the period when the most damage to the turf root system occurs from nematode feeding. This is based on the study of sting nematode feeding habits by Huang & Becker (1997), in which they observed that feeding seemed to be necessary before each juvenile moulting stage and also for egg deposition by females. In the Perth region, the effects of this feeding would not be obvious at the time, due to the mild weather conditions, but would then become evident with the onset of hot weather.

In the depth-distribution study, the detection of adults and juveniles down to 70 cm would suggest that the nematode is capable of reproducing at any depth in the soil profile, although the number of juveniles tended to decrease with depth. There was no evidence of vertical migration from the surface to deeper layers of the soil profile during the hotter months, as occurred with sting nematode in southern California, when the numbers at a depth of 15-30 cm exceeded those in the upper 15 cm (Bekal & Becker 2000a). However, the soil temperatures in the California study were higher than in Perth.

The ability of the southern sting nematode to survive and reproduce so deep in the soil profile has significant implications for turf management. Any control measures applied to the surface, or the removal and replacement of soil, will not prevent reinfestation of the turf by nematodes living deeper in the profile.

Chapter 5. Research: Evaluation of turf cultivars for resistance and tolerance to southern sting nematode

5.1 INTRODUCTION

The southern sting nematode (*Ipibora lolii*) is a major threat to turf grasses in Australia, due to its ability to reproduce on a range of commonly grown species, including couch (*Cynodon dactylon*), hybrid couch (*C. dactylon* x *C. transvaalensis*), kikuyu (*Pennisetum clandestinum*), perennial ryegrass (*Lolium perenne*) and creeping bentgrass (*Agrostis stolonifera*). The nematode can multiply rapidly on sandy soils, and feeding causes severe stunting of the root system and poor turf growth (Siviour & McLeod 1979; Stirling *et al*. 2013).

Although the nematode appears to have a wide host range, the results of studies in pots suggest that there are differences between turf species in the reproduction of *I. lolii*. For example, the population in kikuyu increased to nearly 15 times the initial number after 20 weeks, whereas there was only a two-fold increase in hybrid couch cv. Tifgreen 328 (Stirling *et al*. 2013). In another study, the population on ryegrass increased approximately 25 times the initial number after 90 days, resulting in an 80% reduction in above-ground biomass (Siviour & McLeod 1979).

The reproduction of *I. lolii* and its effect on turf growth have never been assessed in the field, but the response of different turf species and cultivars to the sting nematode (*Belonolaimus longicaudatus*) has been evaluated under field conditions in Florida, USA (Pang *et al*. 2011). This study assessed population densities of *B. longicaudatus* in eight cultivars of couch and three cultivars of seashore paspalum (*Paspalum vaginatum*), and the results showed that couch was a better host of this nematode. Interestingly, there was a decline in *B. longicaudatus* numbers over two years in one of the couch cultivars (TifSport), suggesting that it might have had some level of resistance. In studies with Buffalo/St. Augustinegrass (*Stenotaphrum secundatum*), diploid genotypes were found to be good hosts, although with varying levels of turf damage (Busey *et al*. 1991), whereas polyploid genotypes demonstrated host resistance (Busey *et al*. 1993).

In the Perth region of Western Australia, kikuyu is the turf species of choice on community sports fields, due to its rapid growth, wear tolerance and quick recovery from damage. However, kikuyu is particularly susceptible to southern sting nematode (Stirling *et al*. 2013).

Couch was traditionally grown on sports fields in Perth, but its use has declined because of the invasive nature of kikuyu. However, given the prevalence of southern sting nematode, and the limited reproduction of *I. lolii* and *B. longicaudatus* on some couch cultivars in the studies by Stirling *et al*. (2013) and Pang *et al*. (2011), it is possible that couch is a viable alternative to kikuyu on infested sports fields.

This chapter provides the results of a field experiment that was established to evaluate cultivars of couch and kikuyu for their response to *I. lolii*. The aim was to determine whether any cultivars exhibited either resistance (i.e. the turf's capacity to reduce nematode multiplication and maintain population densities at low levels) or tolerance (i.e. the capacity to grow reasonably well in the presence of high nematode population densities).

5.2 METHODOLOGY

The field experiment was conducted at Lightning Park, Noranda (Lat. -31.87, Long. 115.91). The site has a deep sandy soil profile (>95% sand, 0.05-2.0 mm particle size) with a history of southern sting nematode infestation, the nematode having been introduced on kikuyu planting material during construction in 2007. The experimental plots were located within the irrigated surrounds of the oval to avoid sporting traffic.

Six cultivars were selected for testing: Grand Prix and Wintergreen couch; TifTuf and TifSport hybrid couch; Village Green and common kikuyu. The industry standards were Wintergreen couch and common kikuyu. Village Green is a male-sterile kikuyu that was released in 2009. Grand Prix and TifTurf were becoming commercially available in Western Australia at the time of testing, and TifSport was sourced from Queensland.

The experimental design was a randomised complete block, with five replicates of the six cultivars. There were two separate sets of 30 microplots (0.36 x 0.36 m), one located within an infestation of *I. lolii* and the other in a non-infested area. The relatively small area of the test locations (15 m^2 each) helped to minimise the variability in nematode infestation and watering that occurs in the field.

The test locations were selected based on the health of existing turf and symptoms of nematode damage. Pre-plant nematode counts indicated that all of the infested plots contained *I. lolii* after the existing turf and soil organic matter were removed, with the population densities ranging from 5 to 87 nematodes/200 mL soil. The numbers of other plant-parasitic nematodes were negligible. *I. lolii* was not detected in the non-infested area, except that a few plots had a very low population (1 nematode/200 mL soil) that did not increase after planting. The non-infested plots contained stubby-root (*Paratrichodorus* sp.) and sheath nematodes (*Hemicycliophora* sp.), with population densities ranging from 5 to 100 nematodes/200 mL soil and 10 to 500 nematodes/200 mL soil, respectively. However, both nematodes had declined to negligible numbers two months after planting and did not influence the results.

The test cultivars were initially established in large pots (450 mm diameter and 300 mm depth) from stolons grown on sand, and then transplanted into the field as 30 mm-thick sods. The existing turf and soil organic matter were removed from the plots and replaced with clean sand. A triangular block of turf was then planted on approximately half the area of each microplot (Fig. 5.1), so that its capacity to spread across the bare sand could be assessed. As the experiment was conducted within public open space, grassed buffers were retained for surface stability around the plots.

Figure 5.1. Microplots infested with *Ibipora lolii* as they appeared immediately after planting. Non-infested microplots were located near the base of the light pole in the background.

The turf was typically irrigated daily with approximately 6 mm of water applied through the existing irrigation system on the oval. Additional water was applied to the microplots using a watering can as required in very hot weather. Soluble fertiliser was applied monthly to the microplots with a watering can, at rates of 5 g N/m^2, 1 g P/m^2 and 2 g K/m^2. Mowing was carried out weekly at a height of approximately 20 mm.

The experiment commenced in December 2017 and was assessed at 8 and 17 weeks after planting. The methods of assessment were nematode counts and measurements of root dry weight and total plant biomass. This was done on a 50 mm-diameter soil core taken to a depth of 100 mm, from the same position in each plot. Samples were prepared by separating the soil and plant material using a 2-mm screen. Nematodes were extracted from a 200 mL sub-sample of soil placed on a tray and were retrieved after 2 days using a 38 µm sieve (Whitehead & Hemming 1965). *I. lolii* was counted under a compound microscope at 40X magnification. The plant material was washed thoroughly to remove any remaining soil, and then air dried in paper bags for 4 to 6 weeks. The roots were removed and weighed separately to the other plant material (i.e. rhizomes, stolons, stems and leaves) using a precision balance.

All data were statistically analysed using Minitab 19. The Normality Test using the Ryan-Joiner method (similar to Shapiro-Wilk) found that the data were not normally distributed for the majority of assessments in the nematode infested microplots. This required all data to be log transformed for Analysis of Variance (ANOVA). Levene's Test indicated that equal variances could be assumed for ANOVA testing. Two-Way ANOVA found that the interaction effects of cultivar and nematode infestation, and cultivar and time were not significant. Therefore, One-Way ANOVA was used to determine cultivar differences at a significance level of 0.05.

5.3 RESULTS

There was a rapid increase in the population of *I. lolii* on all cultivars after planting in the infested microplots (Table 5.1). The overall average population density increased from 28 nematodes/200 mL soil before planting, to 188 and 189 nematodes/200 mL soil at 8 and 17 weeks after planting, respectively. Two-way ANOVA found that the difference in nematode population from pre-plant to 8 and 17 weeks was highly significant. Village Green kikuyu and

Grand Prix couch had lower mean numbers of *I. lolii* than the other cultivars after 17 weeks, but the differences were not statistically significant.

Table 5.1. Population densities of *Ibipora lolii* in microplots of six turf grass cultivars before planting and at 8 and 17 weeks after planting.

Cultivar	Mean no. *I. lolii*/200 mL soil [A]		
	Pre-plant	8 Weeks	17 Weeks
TifTuf hybrid couch	22 (1.352)	210 (2.323)	194 (2.288)
TifSport hybrid couch	26 (1.415)	233 (2.368)	214 (2.330)
Grand Prix couch	32 (1.509)	162 (2.210)	129 (2.109)
Wintergreen couch	20 (1.311)	171 (2.232)	185 (2.266)
Village Green kikuyu	21 (1.318)	102 (2.010)	107 (2.031)
Common kikuyu	16 (1.211)	140 (2.145)	205 (2.311)

[A] Data are back-transformed means (n=5) of the transformed data ($\log_{10}$) given in parentheses. Pre-plant means were significantly lower than means at 8 and 17 weeks, but for each sampling date, differences between cultivars were not significant (P<0.05).

In terms of turf growth in the infested microplots, One-way ANOVA identified statistically significant results for root dry weight at 8 weeks (P=0.025) and 17 weeks (P=0.024). Post ANOVA testing with the Tukey method found that Village Green kikuyu was significantly different to Grand Prix couch after 8 weeks and common kikuyu after 17 weeks (Table 5.2). Village Green kikuyu also had the highest total plant biomass at both assessment dates (Table 5.3). However, despite a P-value of 0.046 for the 8-week data, post-ANOVA testing found that there were no statistically significant differences in the pair-wise comparisons.

In the non-infested turf, there were no significant differences between cultivars for total plant biomass or root dry weight. A comparison of the infested and non-infested turf indicates the extent to which *I. lolii* reduced the root dry weight (Fig. 5.2) and total plant biomass (Fig. 5.3). Across all of the cultivars, there was an average of 88% less root dry weight and 53% less total plant biomass in the infested turf after 17 weeks. Two-way ANOVA indicated that the effect of nematode infestation was highly significant for both turf growth parameters.

Table 5.2. Root dry weight in six turf grass cultivars infested with *Ibipora lolii* at 8 and 17 weeks after planting.

Cultivar	Root Dry Weight (mg) [A]	
	8 Weeks	17 Weeks
TifTuf hybrid couch	49 (1.692) ab	30 (1.477) ab
TifSport hybrid couch	25 (1.399) ab	24 (1.376) ab
Grand Prix couch	17 (1.240) b	17 (1.233) ab
Wintergreen couch	25 (1.390) ab	25 (1.397) ab
Village Green kikuyu	62 (1.791) a	37 (1.567) a
Common kikuyu	23 (1.365) ab	14 (1.158) b

[A] Data are back transformed means (n=5) of the transformed data (log_{10}) given in parentheses. In each column, means followed by the same letter are not significantly different (P<0.05).

Table 5.3. Total plant biomass (dry weight of all plant material) in six turf grass cultivars infested with *Ibipora lolii* at 8 and 17 weeks after planting.

Cultivar	Total Plant Biomass (mg) [A]	
	8 Weeks	17 Weeks
TifTuf hybrid couch	1081 (3.034)	805 (2.906)
TifSport hybrid couch	774 (2.889)	641 (2.807)
Grand Prix couch	755 (2.878)	897 (2.953)
Wintergreen couch	755 (2.878)	670 (2.826)
Village Green kikuyu	1202 (3.080)	1143 (3.058)
Common kikuyu	822 (2.915)	631 (2.800)

[A] Data are back transformed means (n=5) of the transformed data (log_{10}) given in parentheses. For each sampling date, the differences between cultivars were not significant (P<0.05).

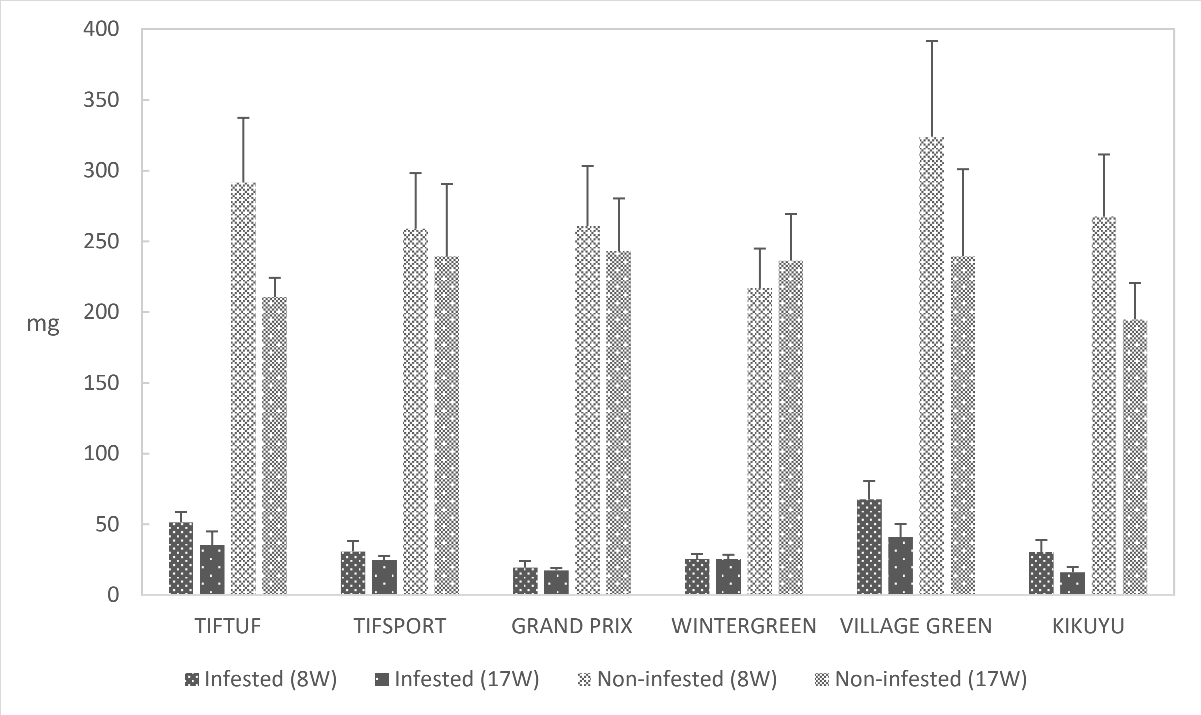

Figure 5.2. Root dry weight (non-transformed means, n=5) in six turf cultivars in microplots infested and not infested with *Ibipora lolii* at 8 & 17 weeks after planting. Error bars represent the standard error of each mean.

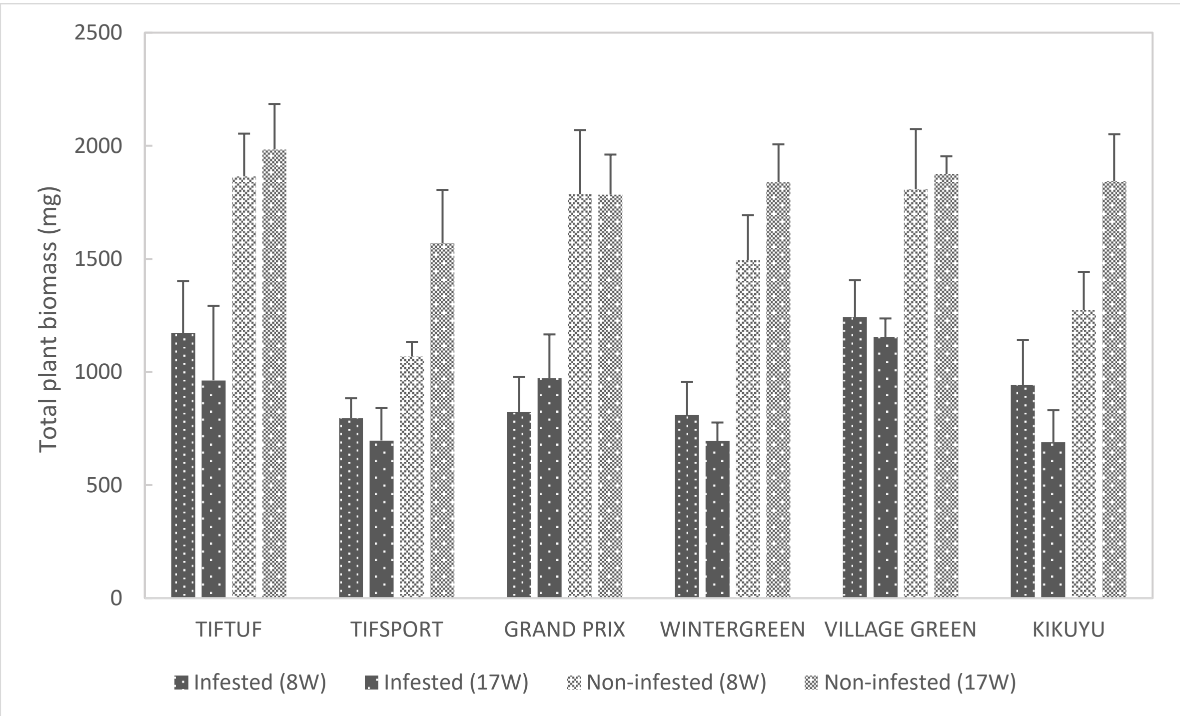

Figure 5.3. Total plant biomass (non-transformed means, n=5) in six turf cultivars in microplots infested and not infested with *Ibipora lolii* at 8 & 17 weeks after planting. Error bars represent the standard error of each mean.

This experiment has demonstrated the severe impact of southern sting nematode on turf establishment and growth. All of the nematode infested turf declined rapidly, suffering a severe moisture stress event at four weeks after planting. An example of Wintergreen couch is shown in Fig. 5.4 A & B. This browning of the turf occurred while the irrigation system was turned off for three days following an unseasonal rain event (≈80 mm) in January. Due to the shallow roots, the infested turf continued to decline for the rest of summer, as shown in Fig. 5.4 C & E. In contrast, the non-infested turf stayed in good condition throughout and increased in coverage of the plots (Fig. 5.4 D & F). All of the cultivars exhibited severe damage from *I. lolii* after 17 weeks (Fig. 5.5).

It was observed that couch infested with *I. lolii* tended to maintain better surface integrity than kikuyu i.e. the ability to prevent the surface from breaking apart. The couch cultivars, TifTuf (Fig. 5.5 A) and Grand Prix (Fig. 5.5 C), were observed to have more consistent turf cover and density across the replicates than TifSport (Fig. 5.5 B), or the industry standard Wintergreen (Fig. 5.5 D). However, there was not a suitable method of objective assessment available to support these observations.

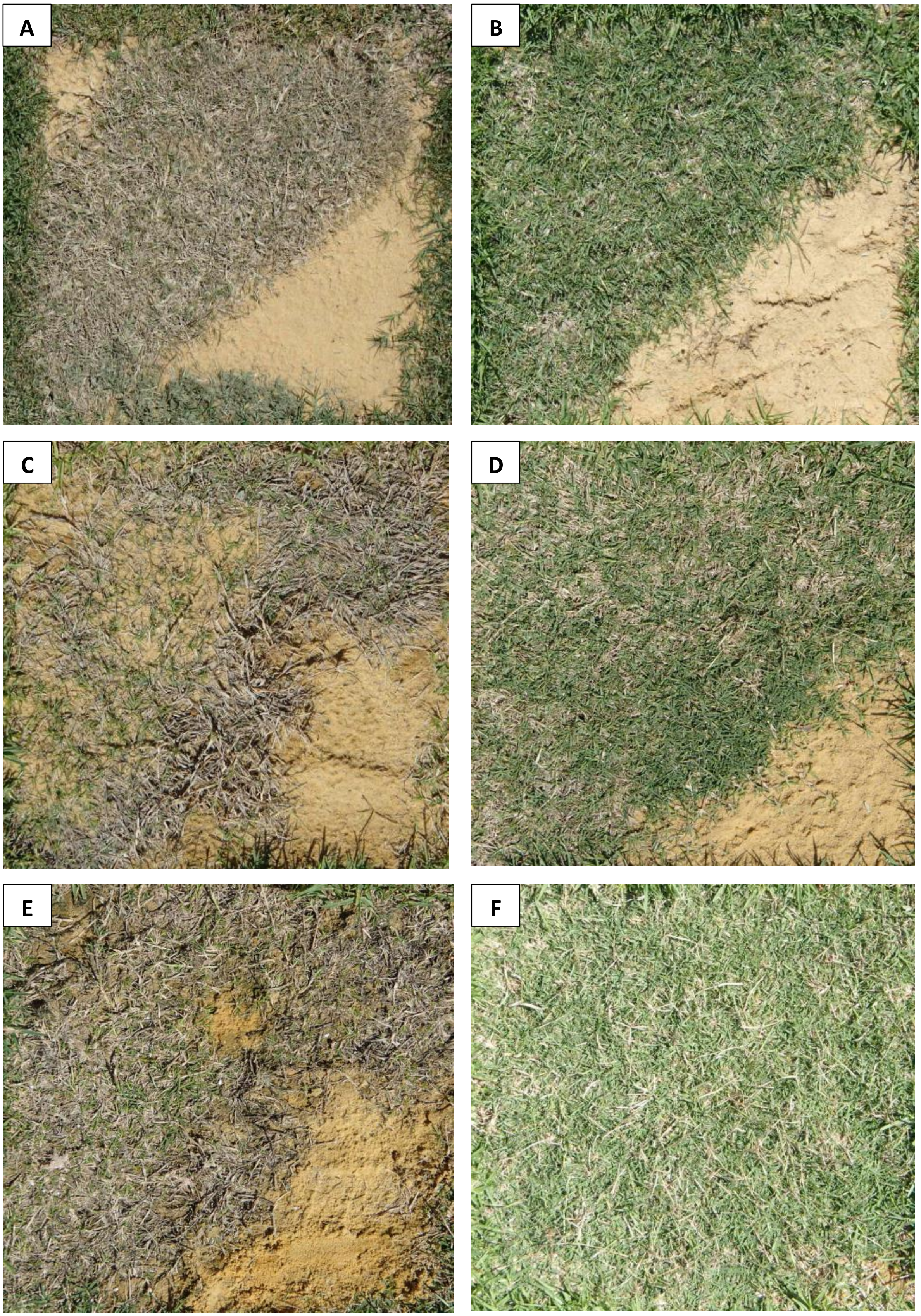

Figure 5.4. Appearance of Wintergreen couch infested (left) and not infested (right) with *Ibipora lolii* at 4 weeks (A & B), 8 weeks (C & D) and 17 weeks (E & F) after planting.

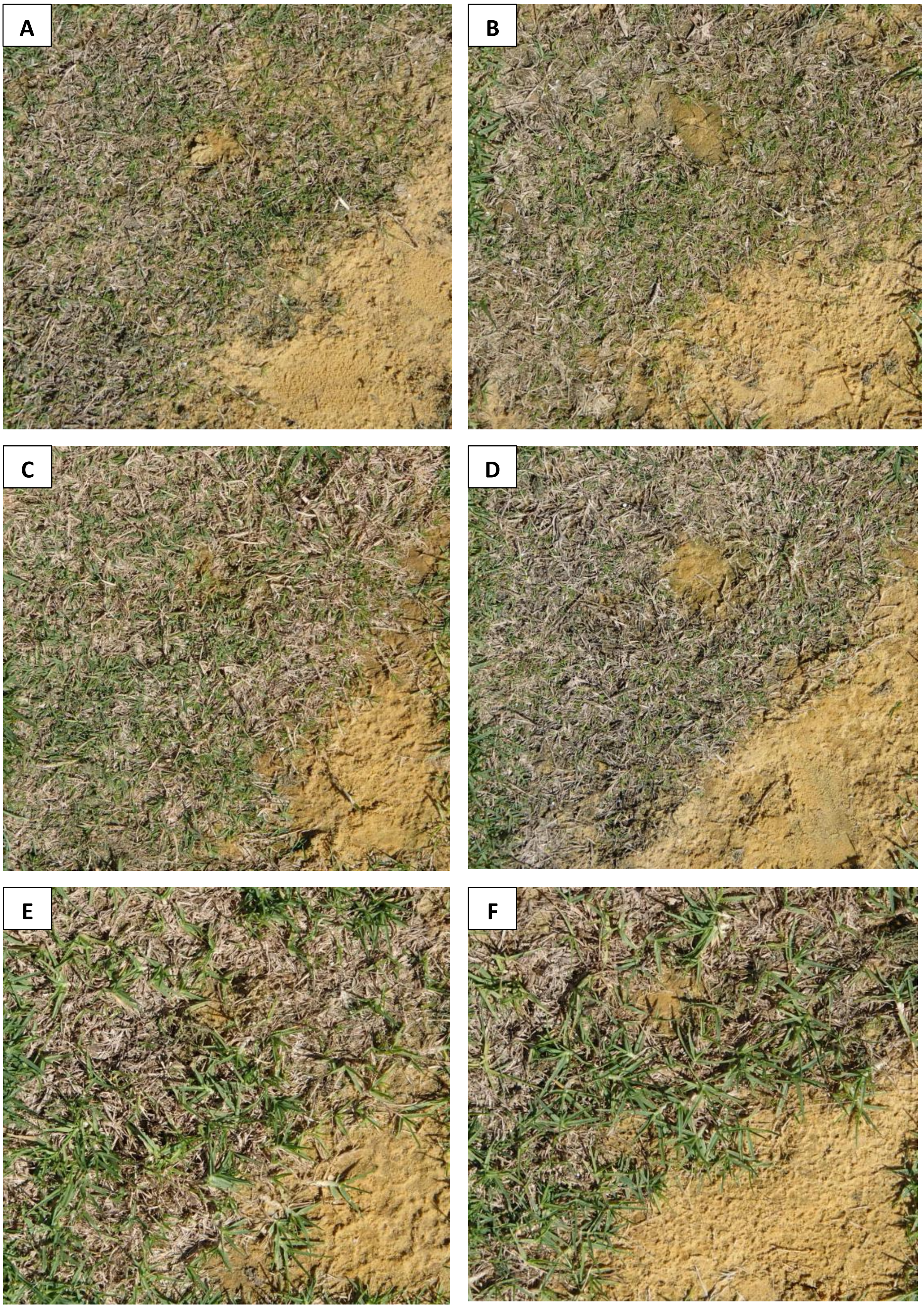

Figure 5.5. Appearance of six turf grass cultivars infested with *Ibipora lolii* at 17 weeks after planting: (A) TifTuf; (B) TifSport; (C) Grand Prix; (D) Wintergreen; (E) Village Green; (F) Kikuyu.

5.4 DISCUSSION

None of the turf cultivars in this experiment demonstrated resistance to southern sting nematode under field conditions. Rapid multiplication of the nematode occurred in all cultivars after planting, and the population densities remained at damaging levels (>100 nematodes/200 mL soil) for a period of four months. There were no significant differences in the reproduction of *I. lolii* on couch and kikuyu cultivars. This was in contrast to the findings of a pot study by Stirling *et al.* (2013), in which common kikuyu had a far greater increase in nematode population than hybrid couch cv. Tifgreen after 20 weeks. TifSport hybrid couch did not exhibit the capacity to reduce *I. lolii* multiplication in the field, as Pang *et al*. (2011) found with *B. longicaudatus*. However, this could be related to the duration of the study, as it took two years for the population of *B. longicaudatus* to decline significantly.

All of the turf cultivars in this experiment exhibited poor tolerance to the high population densities of *I. lolii*, indicated by significant reductions in root growth and total plant biomass. There was an obvious deterioration in the appearance of nematode infested turf, and the capacity of all cultivars to spread across bare sand was severely limited. The timing of the experiment over the summer period meant that the turf was subjected to very stressful conditions during establishment. In more favourable weather, there may have been greater differentiation in nematode tolerance of these cultivars. Similarly, extending the duration of assessment would have given an indication of differences in turf recovery during the winter period.

The apparent difference in surface integrity between couch and kikuyu warrants further investigation. On nematode-infested sports fields, the ability of the turf to prevent the playing surface from breaking apart is an important consideration. The observation of slight differences in the performance of infested couch cultivars should be tested in a longer-term field study of at least 12 months' duration. Objective methods of assessment are needed to evaluate turf coverage, density and strength. Application software can be used to obtain turf colour ratings based on digital photographs, but this method of assessment would not necessarily reflect the integrity of the turf surface.

Given the lack of turf resistance and tolerance to southern sting nematode in this experiment, other management strategies should be considered to reduce turf losses. On unamended sand, the infested turf struggled to survive in typical summer conditions, whereas the non-infested turf established and spread normally. Because infested turf is more prone to moisture stress, due to the shallow root system, it requires daily inspection for symptoms of wilting. In hot weather, supplemental daytime watering will be necessary in addition to the usual nightly irrigation. Ideally, turf should not be planted on unamended sand with an existing infestation of *I. lolii*. The incorporation of an amendment such as compost is essential to improve the water holding capacity in the root zone and give the turf some resilience against the nematode.

Chapter 6. Conclusions

One of the most important findings of the work reported in this thesis was that southern sting nematode was only detected in about half the municipal sports fields in the Perth region. This emphasises the importance of implementing biosecurity measures to prevent the nematode's introduction to the existing non-infested sites and any sports fields established in future. Therefore, turf farms must be monitored regularly and planting material tested prior to being supplied, as this will minimise the risk of spreading the nematode more widely. Considering the interstate movement of turf is common in Australia, it is important that any biosecurity scheme has a national focus, so that infested turf farms are identified wherever they occur.

With the trend toward using free-draining sand for sports turf construction, southern sting nematode will always be a threat, as coarse-textured soils containing little organic matter are ideally suited to this destructive pest. From a nematological perspective, one thing that is not well understood is the capacity of southern sting nematode to spread under its own power or by other means e.g. water movement down slopes; soil adhering to tyres and machinery; and root and soil fragments carried on players' boots. There is a need for research to determine whether the nematode is being moved in these ways, and to investigate control measures that can be implemented to limit the spread from existing infestations.

The survey of municipal sports fields in the Perth region was the first of its kind conducted in Australia. Previous information on nematode distribution was obtained from data collected by certain laboratories from samples that were submitted for nematode testing. The disadvantage of this method is the obvious bias towards sites with nematode problems. For this study, a methodology was developed to provide a very thorough and unbiased survey of nematode distribution in a particular segment of the turf industry, and within a distinct geographical region. There is considerable scope to conduct similar nematode surveys in other segments of the turf industry e.g. golf courses, bowling greens, tennis courts, racecourses, turf farms, parks, schools or even home lawns, not only in the Perth region but also in other parts of Australia.

The population dynamics study showed that in the Perth region, the peak period of southern sting nematode multiplication occurred during the cool and wet conditions in late winter and spring. This knowledge should influence the timing of chemical or non-chemical treatments to suppress nematode activity. Product-specific trial work should be conducted to assess the effectiveness of autumn and early winter treatments, as applications at this time of year may be the best way of limiting nematode multiplication.

This study has revealed two attributes of southern sting nematode that make it extremely challenging to manage. The first is that although peak population densities occur in late winter and spring, the nematode reproduces all-year-round in the Perth region. As a consequence, there is constant feeding pressure on the turf root system, and the population can recover rapidly after treatment. Secondly, the nematode is capable of deep vertical movement in the soil profile (>50 cm). Therefore, any control measures applied to the surface will not prevent reinfestation by nematodes living deeper in the profile. Importantly, this finding also means that southern sting nematode cannot be eradicated from an infested site by removing the existing turf and topsoil. However, it may be possible to eliminate the nematode with an extended fallow period, given that it is a plant parasite that requires roots for feeding. This approach should be investigated.

In the long-term, the use of turf grasses with better resistance or tolerance to southern sting nematode would seem to be the most sustainable management strategy to pursue. The evaluation of couch and kikuyu in this study has provided a good foundation for ongoing research into the selection of more suitable turf species and cultivars. In the work discussed in Chapter 5, the infested turf was under extreme stress from hot weather and high nematode pressure, and this caused rapid turf decline which may have contributed to the inconclusive results. In future field experiments, turf should be established in more favourable conditions, possibly including a soil amendment, and then evaluated over a full year to give the best opportunity for differences to emerge. A further improvement would be to develop objective methods of assessing turf quality to support the observation that couch appeared to have better surface integrity than kikuyu, and to evaluate the apparent differences between couch cultivars.

The turf cultivar study showed that TifSport did not have any resistance to *I. lolii*, even though Pang *et al*. (2011) reported that it might have some level of resistance to *B. longicaudatus*. In the non-infested plots, TifSport exhibited some leaf discolouration, suggesting that it would probably not to be suited to the environmental conditions in Perth. However, couch is a viable alternative to kikuyu on sports fields that are infested with southern sting nematode, and the findings from this study indicate that Grand Prix and TifTuf couch are worthy of further investigation. For passive turf or lawn situations, there is scope to assess other turf species. Seashore paspalum and Queensland blue are two potential candidates, as observations from the nematode survey were that both grew better than kikuyu in localised areas that were infested with southern sting nematode.

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Appendix 1

Nematode Survey Data

Plant-parasitic nematode counts from the survey of municipal sports fields in the Perth region

Council	Site	No. Nematodes/200mL soil								
		STING	DAGGER	STUBBY	SHEATH	SPIRAL	ROOT-KNOT	LESION	RING	SPHAERO NEMA
Joondalup	Chichester North		122	2	12		2			385
	Emerald		684	20	24		8			308
	Forrest		90	8	10					1840
	Iluka			2	2	4				3670
	Korella		54		24					1430
	Marri North		42	8	57		2			390
	Moolanda	29	97	4	36	2	16			
	Ocean Reef		30		74	6	4			1930
	Penistone Upper	49	404	16	4	44				
	Prince Regent	10	564							616
	Timberlane		12	22	38	6				88
	Warrandyte Upper		64	8	48		1			580
Bayswater	Emberson		320	20	380	20	1			
	Frank Drago	3	22	4	10	31		6		
	Halliday	3	102	4	52	40				
	Houghton		332	14	148	32				
	Noranda Soccer	2	86	10	102	46				
Melville	Beasley	10	96	8	44	2				22
	John Connell	5	13	10	71	3	1			4
	Len Shearer		96	17	40	6				
	Marmion		46	10	144	1	1			60
Stirling	Abbett	15	35	1	30	1	1	1		1980
	Aintree	41	20		8	24	12			40
	AS Luketina	34	52	2	2	101	11	10		318

	Birralee Lower	16	30	4	12	16	6			190
	Butlers Lower	14	8		16			8	2	278
	Grindleford South	24		4	9	8	11			
	Karrinyup	51	192	4	2	2	4		2	282
	Linthorne		89	9	40	8	1			
	Princess Lower	60	4	6	61	23	2	2		24
	Richard Guelphi Lower	25	49	2		134				9
	Wordsworth	5	208	16	134	22	4	6		
	Yokine East		238	12	124	4	2			
Armadale	Morgan		212	16	48	18				
	Rushton Upper		176	10	38	92	4			
	William Skeet		30	6	148	156				
Kwinana	Bertram			9	3		24			2
	Kelly		1		3		14			102
Cockburn	Aubin Grove			6	53		20			
	Botany			2	2		6			
	Harmony			2	35			8	2	
	Lucius		4	6	34	2		24	1	905
	Tempest	4	18	2	4		10			122
Belmont	Centenary		2	30	30	44	4		16	
	Forster		22	8	55	35	5			
Canning	Burrendah 2		52	6	74	14				
	Clifton			30	160	76	2			
	Ferndale 3	7	296	6	64	22	8			262
	Hossack Lower	5	128	6	46	26	2	4		
	Queens Park		30	6	60	16	2			
Gosnells	Cassidy	26	432	6	8			14	2	88

	Orange Grove		2	32	138	58				
	Tom Bateman North	28	22	9	7					
	Sutherlands F	48	256			26		1		10
Subiaco	Rosalie East 5	6	29		9		8	4		415
Vic Park	Carlisle	6	111	5	42	1	2	1		79
	Fraser		64	32	84	20	34			
Vincent	Beatty	1	26	12	44	8	12			
	Forrest		58	6	80	10	6			
Mandurah	Coote	66								1070
	Falcon	4	216	4	152				2	2540
	Teranca	7		6	7					
Rockingham	Georgetown E	32	130							850
	Lark Hill Soccer	86				7		11	2	
	Paul Garnett	96						12	1	
	Secret Harbour	31				6		3	8	5
	Stan Twight W	71							1	345
	Tangadee N	59				19	1	12		
Cambridge	Grantham		236	16	14					34
	McLean	9	136	4	12	78				16
Fremantle	Gil Fraser		40	6	102			168		106
	Hilton Mid		14	4	26					214
Kalamunda	Hartfield Rugby		156	16	42	47				
	Scott Cricket	19	171	38	49	1				
South Perth	George Burnett			20	25			4		8
	Morris Mundy		43	31	62	9	3			5
Wanneroo	Alexander Heights	19	52	30	66	6	20			
	Belhaven	21		4	78	22	18			

	Kingsbridge	54			4	4	2	7	2	3
	Kingsway Baseball	58	2	5	7					
	Kingsway Soccer 4	25	26	7	20	1	4			
	Koondoola		220	24	40	14				
	Peridot	16		76	74	122	6			
	Riverlinks			4	6	4	8		2	
	Warradale			32	186	4				
Swan	Ballajura	9	264	14	40	50				
	Ron Jose	11	256	12	48	8	4			4
	Rosher		58	6	160					
	Swan View		106	4	6	12		2		
	Woodlake			4	110	8	18			

Note: Nematodes were extracted from 200 mL of soil placed on a tray and were retrieved after 2 days using a 38 µm sieve (Whitehead & Hemming 1965). Plant-parasitic nematodes were identified to generic level and counted using a compound microscope at 40X magnification.

Summary of nematode survey data

	STING	DAGGER	STUBBY	SHEATH	SPIRAL	ROOT-KNOT	LESION	RING	SPHAERO NEMA
Number of sites detected	46	69	74	80	61	46	21	13	43
Percentage of sites detected	51	77	82	89	68	51	23	14	48
Mean no./200 mL soil	26.5	120.3	11.7	54.5	26.8	7.3	14.7	3.3	503.0
Standard Error of Mean	3.6	16.3	1.4	6.6	4.4	1.1	7.8	1.2	121.5
Minimum no./200 mL soil	1.0	1.0	1.0	2.0	1.0	1.0	1.0	1.0	2.0
Maximum no./200 mL soil	96.0	684.0	76.0	380.0	156.0	34.0	168.0	16.0	3670.0

Note: Total no. of sites = 90

Southern sting nematode counts on selected sports fields

Site	No. per 200 mL soil		
	Good	Moderate	Poor
Prince Regent	25	195	365
Princess Lower	0	226	138
Carlisle	0	258	140
Belhaven	0	84	136
John Connell	0	198	317
Tempest	0	141	171
Noranda Soccer	0	0	47
Ballajura	0	0	277
Hossack Lower	2	104	105
Sutherlands F	237	102	320
Mean	26.4	130.8	201.6
Standard Error	23.5	28.3	34.3

Note: Samples were collected from localised areas that were representative of turf in good, moderate and poor condition.

Root depth assessment on selected sports fields

Site	Root depth (mm)		
	Good	Moderate	Poor
Prince Regent	141	120	73
Princess Lower	173	133	96
Carlisle	121	80	54
Belhaven	153	118	78
John Connell	173	144	101
Tempest	146	116	82
Noranda Soccer	156	123	79
Ballajura	154	115	64
Hossack Lower	185	86	68
Sutherlands F	161	128	73
Mean	156	116	77
Standard Error	5.8	6.2	4.4

Note: Values are the average depth of the longest root measured in each of 5 cores.

Appendix 2

Population Dynamics & Depth Distribution Data

Population density of southern sting nematode at Lightning Park

Date	Plot	No. nematodes/200mL soil (0-10cm)					
		Male	Female	J4	J3	J2	Total
Apr-16	L1	126	80	36	88	23	353
	L2	63	42	39	69	23	236
	L3	35	44	11	36	30	156
	Mean	**75**	**55**	**29**	**64**	**25**	**248**
	SEM	27	12	9	15	2	57
Jun-16	L1	146	98	67	119	64	494
	L2	102	57	32	55	31	277
	L3	195	103	42	62	84	486
	Mean	**148**	**86**	**47**	**79**	**60**	**419**
	SEM	27	15	10	20	15	71
Aug-16	L1	224	176	72	140	200	812
	L2	88	105	37	44	83	357
	L3	272	156	38	60	224	750
	Mean	**195**	**146**	**49**	**81**	**169**	**640**
	SEM	55	21	12	30	44	142
Oct-16	L1	40	66	98	258	232	694
	L2	36	39	66	160	189	490
	L3	34	52	152	348	260	846
	Mean	**37**	**52**	**105**	**255**	**227**	**677**
	SEM	2	8	25	54	21	103
Dec-16	L1	76	88	64	190	166	584
	L2	48	39	33	76	68	264
	L3	68	56	42	92	106	364
	Mean	**64**	**61**	**46**	**119**	**113**	**404**
	SEM	8	14	9	36	29	95
Mar-17	L1	116	82	42	24	14	278
	L2	120	91	41	39	19	310
	L3	198	132	32	38	16	416
	Mean	**145**	**102**	**38**	**34**	**16**	**335**
	SEM	27	15	3	5	1	42
May-17	L1	79	63	54	101	64	361
	L2	61	63	57	67	56	304
	L3	236	110	70	140	150	706
	Mean	**125**	**79**	**60**	**103**	**90**	**457**
	SEM	56	16	5	21	30	126

Jul-17	L1	158	144	43	44	51	440
	L2	142	91	35	30	23	321
	L3	162	152	44	45	65	468
	Mean	**154**	**129**	**41**	**40**	**46**	**410**
	SEM	6	19	3	5	12	45

Oct-17	L1	94	82	50	154	294	674
	L2	83	68	29	87	197	464
	L3	126	134	20	130	254	664
	Mean	**101**	**95**	**33**	**124**	**248**	**601**
	SEM	13	20	9	20	28	68

Dec-17	L1	124	160	90	178	128	680
	L2	108	120	79	80	64	451
	L3	130	100	42	70	96	438
	Mean	**121**	**127**	**70**	**109**	**96**	**523**
	SEM	7	18	15	34	18	79

Mar-18	L1	42	29	41	71	50	233
	L2	37	31	40	77	35	220
	L3	128	58	104	204	150	644
	Mean	**69**	**39**	**62**	**117**	**78**	**366**
	SEM	30	9	21	43	36	139

Apr-18	L1	64	63	67	66	33	293
	L2	105	49	50	53	17	274
	L3	87	48	39	48	17	239
	Mean	**85**	**53**	**52**	**56**	**22**	**269**
	SEM	12	5	8	5	5	16

Depth distribution of southern sting nematode at Lightning Park

Date: Jun-16

Depth	Plot	No. nematodes/200mL soil					
		Male	Female	J4	J3	J2	Total
0-10 cm	L1	146	98	67	119	64	494
	L2	102	57	32	55	31	277
	L3	195	103	42	62	84	486
	Mean	**148**	**86**	**47**	**79**	**60**	**419**
	SEM	27	15	10	20	15	71
10-30 cm	L1	9	15	2	7	14	47
	L2	7	5	11	11	8	42
	L3	23	23	9	6	33	94
	Mean	**13**	**14**	**7**	**8**	**18**	**61**

	SEM	5	5	3	2	8	17
30-50 cm	L1	10	6	2	5	10	33
	L2	20	12	9	7	13	61
	L3	20	13	2	4	19	58
	Mean	**17**	**10**	**4**	**5**	**14**	**51**
	SEM	3	2	2	1	3	9
50-70 cm	L1	18	8	6	7	5	44
	L2	8	7	6	7	12	40
	L3	88	41	5	3	9	146
	Mean	**38**	**19**	**6**	**6**	**9**	**77**
	SEM	25	11	0	1	2	35

Date: Mar-17

Depth	Plot	No. nematodes/200mL soil					
		Male	Female	J4	J3	J2	Total
0-10 cm	L1	116	82	42	24	14	278
	L2	120	91	41	39	19	310
	L3	198	132	32	38	16	416
	Mean	**145**	**102**	**38**	**34**	**16**	**335**
	SEM	27	15	3	5	1	42
10-30 cm	L1	3	3	2	8	9	25
	L2	6	8	0	4	19	37
	L3	4	6	2	2	3	17
	Mean	**4**	**6**	**1**	**5**	**10**	**26**
	SEM	4	2	1	2	5	6
30-50 cm	L1	4	4	3	2	4	17
	L2	13	7	1	3	12	36
	L3	9	11	3	0	3	26
	Mean	**9**	**7**	**2**	**2**	**6**	**26**
	SEM	3	2	1	1	3	5
50-70 cm	L1	12	10	4	3	3	32
	L2	29	26	7	1	5	68
	L3	17	25	3	1	3	49
	Mean	**19**	**20**	**5**	**2**	**4**	**50**
	SEM	5	5	1	1	1	10

Date: Jul-17

Depth	Plot	No. nematodes/200mL soil					
		Male	Female	J4	J3	J2	Total
0-10 cm	L1	158	144	43	44	51	440
	L2	142	91	35	30	23	321
	L3	162	152	44	45	65	468
	Mean	**154**	**129**	**41**	**40**	**46**	**410**
	SEM	6	19	3	5	12	45
10-30 cm	L1	9	8	2	4	10	33

	L2	3	2	1	6	5	17
	L3	26	20	4	4	12	66
	Mean	**13**	**10**	**2**	**5**	**9**	**39**
	SEM	7	5	1	1	2	14
30-50 cm	L1	8	12	2	4	4	30
	L2	5	4	2	4	5	20
	L3	7	5	2	1	7	22
	Mean	**7**	**7**	**2**	**3**	**5**	**24**
	SEM	1	3	0	1	1	3
50-70 cm	L1	11	15	4	2	2	34
	L2	5	3	4	2	6	20
	L3	8	7	4	0	0	19
	Mean	**8**	**8**	**4**	**1**	**3**	**24**
	SEM	2	4	0	1	2	5

Date: **Mar-18**

Depth	Plot	No. nematodes/200mL soil					
		Male	Female	J4	J3	J2	Total
0-10 cm	L1	42	29	41	71	50	233
	L2	37	31	40	77	35	220
	L3	128	58	104	204	150	644
	Mean	**69**	**39**	**62**	**117**	**78**	**366**
	SEM	30	9	21	43	36	139
10-30 cm	L1	6	14	7	21	35	83
	L2	15	9	5	4	20	53
	L3	21	17	30	35	90	193
	Mean	**14**	**13**	**14**	**20**	**48**	**110**
	SEM	4	2	8	9	21	43
30-50 cm	L1	18	6	14	12	12	62
	L2	14	11	5	3	9	42
	L3	4	3	11	3	10	31
	Mean	**12**	**7**	**10**	**6**	**10**	**45**
	SEM	4	2	3	3	1	9
50-70 cm	L1	10	4	5	3	4	26
	L2	27	20	11	3	23	84
	L3	16	2	5	1	1	25
	Mean	**18**	**9**	**7**	**2**	**9**	**45**
	SEM	5	6	2	1	7	20

Note: Nematodes were extracted from 200 mL of soil placed on a tray and were retrieved after 2 days using a 38 µm sieve (Whitehead & Hemming 1965). Southern sting nematodes were identified and counted in life stages using a compound microscope at 40X magnification.

Population density of southern sting nematode at Charles Riley Reserve

Date	Plot	No. nematodes/200mL soil (0-10cm)					
		Male	Female	J4	J3	J2	Total
Apr-16	CR1	3	1	2	10	8	24
	CR2	20	13	20	35	24	112
	CR3	4	4	4	6	2	20
	Mean	**9**	**6**	**9**	**17**	**11**	**52**
	SEM	6	4	6	9	7	30
Jun-16	CR1	17	6	3	7	1	34
	CR2	28	15	5	18	6	72
	CR3	8	2	3	2	4	19
	Mean	**18**	**8**	**4**	**9**	**4**	**42**
	SEM	6	4	1	5	1	16
Aug-16	CR1	23	12	0	16	21	72
	CR2	43	27	11	26	23	130
	CR3	2	5	3	6	7	23
	Mean	**23**	**15**	**5**	**16**	**17**	**75**
	SEM	12	6	3	6	5	31
Oct-16	CR1	4	2	13	14	10	43
	CR2	28	30	39	51	49	197
	CR3	3	10	2	8	12	35
	Mean	**12**	**14**	**18**	**24**	**24**	**92**
	SEM	8	8	11	13	13	53
Dec-16	CR1	0	5	2	6	7	20
	CR2	14	8	3	7	4	36
	CR3	1	1	0	3	1	6
	Mean	**5**	**5**	**2**	**5**	**4**	**21**
	SEM	5	2	1	1	2	9
Feb-17	CR1	0	1	2	23	8	34
	CR2	0	4	9	18	14	45
	CR3	1	0	0	3	1	5
	Mean	**0**	**2**	**4**	**15**	**8**	**28**
	SEM	0	1	3	6	4	12
May-17	CR1	1	3	9	12	5	30
	CR2	19	15	16	29	16	95
	CR3	5	4	2	2	0	13
	Mean	**8**	**7**	**9**	**14**	**7**	**46**
	SEM	5	4	4	8	5	25

Jul-17	CR1	10	7	1	8	10	36
	CR2	29	20	18	18	17	102
	CR3	3	6	2	7	5	23
	Mean	**14**	**11**	**7**	**11**	**11**	**54**
	SEM	8	5	6	4	4	24

Oct-17	CR1	1	3	6	6	8	24
	CR2	26	18	8	19	34	105
	CR3	1	1	1	1	6	10
	Mean	**9**	**7**	**5**	**9**	**16**	**46**
	SEM	8	5	2	5	9	30

Dec-17	CR1	2	7	8	3	6	26
	CR2	9	27	15	27	13	91
	CR3	1	4	2	1	3	11
	Mean	**4**	**13**	**8**	**10**	**7**	**43**
	SEM	3	7	4	8	3	25

Mar-18	CR1	1	3	2	2	0	8
	CR2	16	16	6	12	6	56
	CR3	0	1	0	0	1	2
	Mean	**6**	**7**	**3**	**5**	**2**	**22**
	SEM	5	5	2	4	2	17

May-18	CR1	5	2	8	8	8	31
	CR2	6	14	15	23	18	76
	CR3	0	0	0	1	1	2
	Mean	**4**	**5**	**8**	**11**	**9**	**36**
	SEM	2	4	4	6	5	22

Depth distribution of southern sting nematode at Charles Riley Reserve

Date: Jun-16

Depth	Plot	No. nematodes/200mL soil					
		Male	Female	J4	J3	J2	Total
0-10 cm	CR1	17	6	3	7	1	34
	CR2	28	15	5	18	6	72
	CR3	8	2	3	2	4	19
	Mean	**18**	**8**	**4**	**9**	**4**	**42**
	SEM	6	4	1	5	1	16
10-30 cm	CR1	3	3	0	2	2	10
	CR2	10	6	0	4	8	28
	CR3	2	4	3	1	1	11
	Mean	**5**	**4**	**1**	**2**	**4**	**16**
	SEM	3	1	1	1	2	6

30-50 cm	CR1	11	5	2	2	2	22
	CR2	14	3	0	1	2	20
	CR3	3	1	1	0	0	5
	Mean	**9**	**3**	**1**	**1**	**1**	**16**
	SEM	3	2	1	1	1	5
50-70 cm	CR1	40	6	3	6	1	56
	CR2	19	11	0	4	0	34
	CR3	3	3	0	0	0	6
	Mean	**21**	**7**	**1**	**3**	**0**	**32**
	SEM	11	2	1	2	0	10

Date: Feb-17

Depth	Plot	No. nematodes/200mL soil					
		Male	Female	J4	J3	J2	Total
0-10 cm	CR1	0	1	2	23	8	34
	CR2	0	4	9	18	14	45
	CR3	1	0	0	3	1	5
	Mean	**0**	**2**	**4**	**15**	**8**	**28**
	SEM	0	1	3	6	4	12
10-30 cm	CR1	2	2	4	5	2	15
	CR2	2	4	1	0	1	8
	CR3	1	1	0	0	0	2
	Mean	**2**	**2**	**2**	**2**	**1**	**8**
	SEM	0	1	1	2	1	4
30-50 cm	CR1	7	0	1	3	0	11
	CR2	5	2	0	2	1	10
	CR3	3	1	0	0	0	4
	Mean	**5**	**1**	**0**	**2**	**0**	**8**
	SEM	2	1	0	1	0	2
50-70 cm	CR1	15	2	1	0	2	20
	CR2	8	4	0	1	0	13
	CR3	1	0	0	0	0	1
	Mean	**8**	**2**	**0**	**0**	**1**	**11**
	SEM	4	2	0	0	1	6

Date: Jul-17

Depth	Plot	No. nematodes/200mL soil					
		Male	Female	J4	J3	J2	Total
0-10 cm	CR1	10	7	1	8	10	36
	CR2	29	20	18	18	17	102
	CR3	3	6	2	7	5	23
	Mean	**14**	**11**	**7**	**11**	**11**	**54**
	SEM	8	5	6	4	4	24

10-30 cm	CR1	2	6	1	3	3	15
	CR2	3	1	1	1	3	9
	CR3	0	0	0	1	0	1
	Mean	**2**	**2**	**1**	**2**	**2**	**8**
	SEM	1	2	0	1	1	4
30-50 cm	CR1	12	5	0	0	0	17
	CR2	4	3	1	2	2	12
	CR3	0	0	0	0	1	1
	Mean	**5**	**3**	**0**	**1**	**1**	**10**
	SEM	4	1	0	1	1	5
50-70 cm	CR1	4	4	1	0	1	10
	CR2	9	4	0	1	2	16
	CR3	1	0	0	0	0	1
	Mean	**5**	**3**	**0**	**0**	**1**	**9**
	SEM	2	1	0	0	1	4

Date: **Mar-18**

Depth	Plot	No. nematodes/200mL soil					
		Male	Female	J4	J3	J2	Total
0-10 cm	CR1	1	3	2	2	0	8
	CR2	16	16	6	12	6	56
	CR3	0	1	0	0	1	2
	Mean	**6**	**7**	**3**	**5**	**2**	**22**
	SEM	5	5	2	4	2	17
10-30 cm	CR1	0	1	1	1	2	5
	CR2	2	0	2	3	3	10
	CR3	0	0	0	0	0	0
	Mean	**1**	**0**	**1**	**1**	**2**	**5**
	SEM	1	0	1	1	1	3
30-50 cm	CR1	9	4	2	1	1	17
	CR2	5	1	2	1	1	10
	CR3	0	0	0	0	0	0
	Mean	**5**	**2**	**1**	**1**	**1**	**9**
	SEM	3	1	1	0	0	5
50-70 cm	CR1	27	1	2	0	0	30
	CR2	12	1	1	0	2	16
	CR3	0	0	0	1	0	1
	Mean	**13**	**1**	**1**	**0**	**1**	**16**
	SEM	8	0	1	0	1	8

Note: Nematodes were extracted from 200 mL of soil placed on a tray and were retrieved after 2 days using a 38 µm sieve (Whitehead & Hemming 1965). Southern sting nematodes were identified and counted in life stages using a compound microscope at 40X magnification.

Appendix 3

Turf Cultivar Evaluation Data & Statistical Analysis

Southern sting nematode counts in the infested microplots of six turf grass cultivars

Cultivar	Rep	No. nematodes/200 mL soil Pre-plant	8 weeks	17 weeks	Log Pre-plant	Log 8 weeks	Log 17 weeks
TifTuf	1	23	245	463	1.362	2.390	2.665
TifTuf	2	13	199	193	1.114	2.299	2.286
TifTuf	3	13	451	203	1.114	2.654	2.307
TifTuf	4	48	154	146	1.681	2.188	2.163
TifTuf	5	31	122	105	1.491	2.087	2.021
TifSport	1	27	290	224	1.431	2.463	2.351
TifSport	2	10	306	202	1.000	2.485	2.305
TifSport	3	17	195	191	1.230	2.289	2.281
TifSport	4	74	144	247	1.869	2.159	2.393
TifSport	5	35	277	208	1.544	2.443	2.318
Grand Prix	1	27	132	230	1.431	2.121	2.361
Grand Prix	2	13	122	102	1.114	2.087	2.009
Grand Prix	3	44	221	115	1.643	2.345	2.061
Grand Prix	4	58	103	161	1.763	2.015	2.206
Grand Prix	5	39	306	81	1.591	2.485	1.910
Wintergreen	1	5	92	119	0.699	1.966	2.075
Wintergreen	2	24	263	308	1.380	2.420	2.489
Wintergreen	3	15	160	151	1.176	2.203	2.179
Wintergreen	4	87	109	215	1.940	2.037	2.332
Wintergreen	5	23	344	179	1.362	2.537	2.254
Village Green	1	13	136	159	1.114	2.135	2.202
Village Green	2	14	80	128	1.146	1.905	2.107
Village Green	3	35	51	112	1.544	1.704	2.049
Village Green	4	21	142	108	1.322	2.152	2.035
Village Green	5	29	142	58	1.462	2.152	1.760
Kikuyu	1	17	48	416	1.230	1.685	2.619
Kikuyu	2	21	78	369	1.322	1.893	2.567
Kikuyu	3	6	233	303	0.778	2.368	2.481
Kikuyu	4	14	306	117	1.146	2.485	2.067
Kikuyu	5	38	197	66	1.580	2.294	1.820

Note: Nematodes were extracted from 200 mL of soil placed on a tray and were retrieved after 2 days using a 38 µm sieve (Whitehead & Hemming 1965). Southern sting nematodes were identified and counted using a compound microscope at 40X magnification.

Southern sting nematode counts in the non-infested microplots of six turf grass cultivars

		No. nematodes/200 mL soil		
Cultivar	Rep	Pre-plant	8 weeks	17 weeks
TifTuf	1	0	0	0
TifTuf	2	0	0	0
TifTuf	3	0	0	0
TifTuf	4	0	0	0
TifTuf	5	0	0	0
TifSport	1	0	0	0
TifSport	2	0	0	0
TifSport	3	0	0	0
TifSport	4	0	0	0
TifSport	5	0	0	0
Grand Prix	1	0	0	0
Grand Prix	2	1	0	1
Grand Prix	3	0	0	0
Grand Prix	4	0	0	0
Grand Prix	5	0	0	0
Wintergreen	1	0	0	0
Wintergreen	2	0	0	0
Wintergreen	3	0	0	0
Wintergreen	4	0	0	0
Wintergreen	5	0	0	0
Village Green	1	0	0	0
Village Green	2	0	0	0
Village Green	3	1	1	0
Village Green	4	0	0	0
Village Green	5	0	0	0
Kikuyu	1	1	3	0
Kikuyu	2	0	0	0
Kikuyu	3	0	0	0
Kikuyu	4	1	0	0
Kikuyu	5	0	0	0

Note: Nematodes were extracted from 200 mL of soil placed on a tray and were retrieved after 2 days using a 38 µm sieve (Whitehead & Hemming 1965). Southern sting nematodes were identified and counted using a compound microscope at 40X magnification.

Total plant biomass in the infested microplots of six turf grass cultivars

		Total Plant Biomass (mg)			
Cultivar	Rep	8 weeks	17 weeks	log 8 weeks	log 17 weeks
TifTuf	1	1369	2258	3.136	3.354
TifTuf	2	680	509	2.833	2.707
TifTuf	3	1872	493	3.272	2.693
TifTuf	4	1287	852	3.110	2.930
TifTuf	5	656	702	2.817	2.846
TifSport	1	842	529	2.925	2.723
TifSport	2	525	336	2.720	2.526
TifSport	3	819	720	2.913	2.857
TifSport	4	1068	1195	3.029	3.077
TifSport	5	724	706	2.860	2.849
Grand Prix	1	395	979	2.597	2.991
Grand Prix	2	511	474	2.708	2.676
Grand Prix	3	950	1662	2.978	3.221
Grand Prix	4	1110	803	3.045	2.905
Grand Prix	5	1149	942	3.060	2.974
Wintergreen	1	589	393	2.770	2.594
Wintergreen	2	1069	712	3.029	2.852
Wintergreen	3	447	812	2.650	2.910
Wintergreen	4	1231	870	3.090	2.940
Wintergreen	5	712	685	2.852	2.836
Village Green	1	896	969	2.952	2.986
Village Green	2	989	1091	2.995	3.038
Village Green	3	1529	1021	3.184	3.009
Village Green	4	1070	1302	3.029	3.115
Village Green	5	1730	1391	3.238	3.143
Kikuyu	1	833	1098	2.921	3.041
Kikuyu	2	1312	938	3.118	2.972
Kikuyu	3	915	406	2.961	2.609
Kikuyu	4	1384	392	3.141	2.593
Kikuyu	5	272	610	2.435	2.785

Note: Total plant biomass refers to the dry weight of all plant material in a 50 mm-diameter core sample measured using a precision balance.

Total plant biomass in the non-infested microplots of six turf grass cultivars

		Total Plant Biomass (mg)			
Cultivar	Rep	8 weeks	17 weeks	log 8 weeks	log 17 weeks
TifTuf	1	2325	1292	3.366	3.111
TifTuf	2	1387	1929	3.142	3.285
TifTuf	3	1819	2194	3.260	3.341
TifTuf	4	1527	1991	3.184	3.299
TifTuf	5	2265	2513	3.355	3.400
TifSport	1	907	1822	2.958	3.261
TifSport	2	1189	1487	3.075	3.172
TifSport	3	1177	2335	3.071	3.368
TifSport	4	1156	1182	3.063	3.073
TifSport	5	913	1024	2.960	3.010
Grand Prix	1	2117	1662	3.326	3.221
Grand Prix	2	1058	2050	3.024	3.312
Grand Prix	3	1452	1140	3.162	3.057
Grand Prix	4	1616	1989	3.208	3.299
Grand Prix	5	2690	2080	3.430	3.318
Wintergreen	1	882	1583	2.945	3.199
Wintergreen	2	1310	1450	3.117	3.161
Wintergreen	3	1900	1838	3.279	3.264
Wintergreen	4	1946	1908	3.289	3.281
Wintergreen	5	1439	2418	3.158	3.383
Village Green	1	2105	1731	3.323	3.238
Village Green	2	2623	1723	3.419	3.236
Village Green	3	1487	2042	3.172	3.310
Village Green	4	1063	2084	3.027	3.319
Village Green	5	1762	1799	3.246	3.255
Kikuyu	1	1592	2510	3.202	3.400
Kikuyu	2	775	1278	2.889	3.107
Kikuyu	3	1097	1701	3.040	3.231
Kikuyu	4	1191	2064	3.076	3.315
Kikuyu	5	1709	1663	3.233	3.221

Note: Total plant biomass refers to the dry weight of all plant material in a 50 mm-diameter core sample measured using a precision balance.

Root dry weight in the infested microplots of six turf grass cultivars

		Root Dry Weight (mg)			
Treatment	Rep	8 weeks	17 weeks	log 8 weeks	log 17 weeks
TifTuf	1	63	64	1.799	1.806
TifTuf	2	36	30	1.556	1.477
TifTuf	3	69	12	1.839	1.079
TifTuf	4	56	50	1.748	1.699
TifTuf	5	33	21	1.519	1.322
TifSport	1	50	17	1.699	1.230
TifSport	2	25	23	1.398	1.362
TifSport	3	33	32	1.519	1.505
TifSport	4	40	32	1.602	1.505
TifSport	5	6	19	0.778	1.279
Grand Prix	1	28	17	1.447	1.230
Grand Prix	2	12	14	1.079	1.146
Grand Prix	3	13	24	1.114	1.380
Grand Prix	4	11	17	1.041	1.230
Grand Prix	5	33	15	1.519	1.176
Wintergreen	1	38	26	1.580	1.415
Wintergreen	2	27	28	1.431	1.447
Wintergreen	3	19	35	1.279	1.544
Wintergreen	4	19	19	1.279	1.279
Wintergreen	5	24	20	1.380	1.301
Village Green	1	29	19	1.462	1.279
Village Green	2	56	76	1.748	1.881
Village Green	3	102	40	2.009	1.602
Village Green	4	60	32	1.778	1.505
Village Green	5	91	37	1.959	1.568
Kikuyu	1	35	31	1.544	1.491
Kikuyu	2	56	15	1.748	1.176
Kikuyu	3	36	15	1.556	1.176
Kikuyu	4	19	8	1.279	0.903
Kikuyu	5	5	11	0.699	1.041

Note: Dry weight of roots in a 50 mm-diameter core sample measured using a precision balance.

Root dry weight in the non-infested microplots of six turf grass cultivars

		Root Dry Weight (mg)			
Treatment	Rep	8 weeks	17 weeks	log 8 weeks	log 17 weeks
TifTuf	1	435	209	2.638	2.320
TifTuf	2	172	263	2.236	2.420
TifTuf	3	282	199	2.450	2.299
TifTuf	4	226	198	2.354	2.297
TifTuf	5	343	184	2.535	2.265
TifSport	1	210	352	2.322	2.547
TifSport	2	414	371	2.617	2.569
TifSport	3	230	183	2.362	2.262
TifSport	4	207	176	2.316	2.246
TifSport	5	234	114	2.369	2.057
Grand Prix	1	270	253	2.431	2.403
Grand Prix	2	160	377	2.204	2.576
Grand Prix	3	171	190	2.233	2.279
Grand Prix	4	332	234	2.521	2.369
Grand Prix	5	372	162	2.571	2.210
Wintergreen	1	244	143	2.387	2.155
Wintergreen	2	131	232	2.117	2.365
Wintergreen	3	258	293	2.412	2.467
Wintergreen	4	278	323	2.444	2.509
Wintergreen	5	175	191	2.243	2.281
Village Green	1	347	459	2.540	2.662
Village Green	2	531	277	2.725	2.442
Village Green	3	388	203	2.589	2.307
Village Green	4	196	139	2.292	2.143
Village Green	5	158	119	2.199	2.076
Kikuyu	1	315	264	2.498	2.422
Kikuyu	2	144	121	2.158	2.083
Kikuyu	3	388	184	2.589	2.265
Kikuyu	4	194	239	2.288	2.378
Kikuyu	5	297	166	2.473	2.220

Note: Dry weight of roots in a 50 mm-diameter core sample measured using a precision balance.

Summary of results for total plant biomass and root dry weight

Mean Total Plant Biomass

Cultivar	Infested (8W)	Infested (17W)	Non-infested (8W)	Non-infested (17W)
TIFTUF	1172.8	962.8	1864.6	1983.8
TIFSPORT	795.6	697.2	1068.4	1570.0
GRAND PRIX	823.0	972.0	1786.6	1784.2
WINTERGREEN	809.6	694.4	1495.4	1839.4
VILLAGE GREEN	1242.8	1154.8	1808.0	1875.8
KIKUYU	943.2	688.8	1272.8	1843.2
Overall Average	**964.5**	**861.7**	**1549.3**	**1816.1**

Standard Error Total Plant Biomass

Cultivar	Infested (8W)	Infested (17W)	Non-infested (8W)	Non-infested (17W)
TIFTUF	229.0	330.0	189.0	201.0
TIFSPORT	88.1	143.0	64.9	235.0
GRAND PRIX	156.0	194.0	283.0	177.0
WINTERGREEN	147.0	82.4	198.0	167.0
VILLAGE GREEN	163.0	81.8	266.0	77.8
KIKUYU	199.0	142.0	170.0	208.0

Mean Root Dry Weight

Cultivar	Infested (8W)	Infested (17W)	Non-infested (8W)	Non-infested (17W)
TIFTUF	51.4	35.4	291.6	210.6
TIFSPORT	30.8	24.6	259.0	239.2
GRAND PRIX	19.4	17.4	261.0	243.2
WINTERGREEN	25.4	25.6	217.2	236.4
VILLAGE GREEN	67.6	40.8	324.0	239.4
KIKUYU	30.2	16.0	267.6	194.8
Overall Average	**37.5**	**26.6**	**270.1**	**227.3**

Standard Error Root Dry Weight

Cultivar	Infested (8W)	Infested (17W)	Non-infested (8W)	Non-infested (17W)
TIFTUF	7.2	9.5	45.8	13.7
TIFSPORT	7.4	3.2	39.1	51.4
GRAND PRIX	4.6	1.8	42.3	37.1
WINTERGREEN	3.5	2.9	27.7	32.8
VILLAGE GREEN	13.1	9.5	67.6	61.5
KIKUYU	8.6	4.0	43.8	25.6

Statistical analysis

TWO-WAY ANOVA

Nematode Infested (log) versus Cultivar, Time

Factor	Type	Levels	Values
Cultivar	Fixed	6	Grand Prix, Kikuyu, TifSport, TifTuf, Village Green, Wintergreen
Time	Fixed	3	17 weeks, 8 weeks, Pre-plant

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cultivar	5	0.5634	0.11269	1.78	0.127
Time	2	14.9951	7.49754	118.75	0.000
Cultivar*Time	10	0.4808	0.04808	0.76	0.665
Error	72	4.5458	0.06314		
Total	89	20.5851			

Total Plant Biomass Infested (log) versus Cultivar, Time

Factor	Type	Levels	Values
Cultivar	Fixed	6	Grand Prix, Kikuyu, TifSport, TifTuf, Village Green, Wintergreen
Time	Fixed	2	17 weeks, 8 weeks

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cultivar	5	0.38353	0.07671	2.07	0.085
Time	1	0.04365	0.04365	1.18	0.283
Cultivar*Time	5	0.06949	0.01390	0.38	0.863
Error	48	1.77529	0.03699		
Total	59	2.27196			

Root Dry Weight Infested (log) versus Cultivar, Time

Factor	Type	Levels	Values
Cultivar	Fixed	6	Grand Prix, Kikuyu, TifSport, TifTuf, Village Green, Wintergreen
Time	Fixed	2	17 weeks, 8 weeks

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cultivar	5	1.5474	0.30948	5.73	0.000
Time	1	0.1872	0.18719	3.46	0.069
Cultivar*Time	5	0.1640	0.03281	0.61	0.695
Error	48	2.5932	0.05403		
Total	59	4.4919			

Total Plant Biomass 8 weeks (log) versus Cultivar, Nematode

Factor	Type	Levels	Values
Cultivar	Fixed	6	Grand Prix, Kikuyu, TifSport, TifTuf, Village Green, Wintergreen
Nematode	Fixed	2	Infested, Non-infested

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cultivar	5	0.33027	0.06605	2.43	0.048
Nematode	1	0.73261	0.73261	26.96	0.000
Cultivar*Nematode	5	0.08572	0.01714	0.63	0.677
Error	48	1.30455	0.02718		
Total	59	2.45315			

Total Plant Biomass 17 weeks (log) versus Cultivar, Nematode

Factor	Type	Levels	Values
Cultivar	Fixed	6	Grand Prix, Kikuyu, TifSport, TifTuf, Village Green, Wintergreen
Nematode	Fixed	2	Infested, Non-infested

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cultivar	5	0.1920	0.03841	1.64	0.169
Nematode	1	1.9065	1.90654	81.22	0.000
Cultivar*Nematode	5	0.1030	0.02059	0.88	0.504
Error	48	1.1268	0.02347		
Total	59	3.3283			

Root Dry Weight 8 weeks (log) versus Cultivar, Nematode

Factor	Type	Levels	Values
Cultivar	Fixed	6	Grand Prix, Kikuyu, TifSport, TifTuf, Village Green, Wintergreen
Nematode	Fixed	2	Infested, Non-infested

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cultivar	5	0.7992	0.1598	3.21	0.014
Nematode	1	12.8122	12.8122	257.39	0.000
Cultivar*Nematode	5	0.4020	0.0804	1.62	0.174
Error	48	2.3893	0.0498		
Total	59	16.4027			

Root Dry Weight 17 weeks (log) versus Cultivar, Nematode

Factor	Type	Levels	Values
Cultivar	Fixed	6	Grand Prix, Kikuyu, TifSport, TifTuf, Village Green, Wintergreen
Nematode	Fixed	2	Infested, Non-infested

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cultivar	5	0.3296	0.0659	2.08	0.084
Nematode	1	13.8787	13.8787	438.26	0.000
Cultivar*Nematode	5	0.2720	0.0544	1.72	0.149
Error	48	1.5201	0.0317		
Total	59	16.0004			

ONE-WAY ANOVA

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Nematode Infested Pre-Plant (log) versus Cultivar

Factor	Levels	Values
Cultivar	6	Grand Prix, Kikuyu, TifSport, TifTuf, Village Green, Wintergreen

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cultivar	5	0.2556	0.05112	0.56	0.731

Error	24	2.2011	0.09171
Total	29	2.4567	

Nematode Infested 8 weeks (log) versus Cultivar

Factor	Levels	Values
Cultivar	6	Grand Prix, Kikuyu, TifSport, TifTuf, Village Green, Wintergreen

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cultivar	5	0.4128	0.08257	1.54	0.214
Error	24	1.2845	0.05352		
Total	29	1.6973			

Nematode Infested 17 weeks (log) versus Cultivar

Factor	Levels	Values
Cultivar	6	Grand Prix, Kikuyu, TifSport, TifTuf, Village Green, Wintergreen

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cultivar	5	0.3758	0.07517	1.70	0.173
Error	24	1.0602	0.04418		
Total	29	1.4360			

Total Plant Biomass Infested 8 weeks (log) versus Cultivar

Factor	Levels	Values
Cultivar	6	Grand Prix, Kikuyu, TifSport, TifTuf, Village Green, Wintergreen

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cultivar	5	0.1949	0.03897	1.03	0.424
Error	24	0.9098	0.03791		
Total	29	1.1047			

Total Plant Biomass Infested 17 weeks (log) versus Cultivar

Factor	Levels	Values
Cultivar	6	Grand Prix, Kikuyu, TifSport, TifTuf, Village Green, Wintergreen

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cultivar	5	0.2582	0.05163	1.43	0.249
Error	24	0.8655	0.03606		
Total	29	1.1236			

Root Dry Weight Infested 8 weeks (log) versus Cultivar

Factor	Levels	Values
Cultivar	6	Grand Prix, Kikuyu, TifSport, TifTuf, Village Green, Wintergreen

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cultivar	5	1.137	0.22737	3.15	0.025
Error	24	1.732	0.07217		
Total	29	2.869			

Grouping Information Using the Tukey Method and 95% Confidence

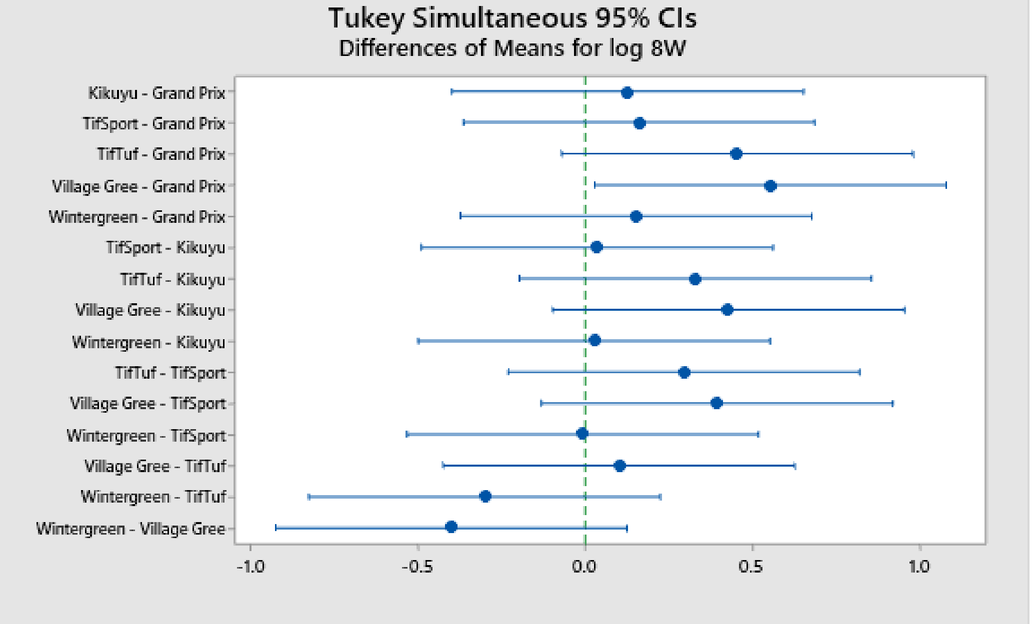
Cultivar	N	Mean	Grouping	
Village Green	5	1.7913	A	
TifTuf	5	1.6922	A	B
TifSport	5	1.399	A	B
Wintergreen	5	1.3898	A	B
Kikuyu	5	1.365	A	B
Grand Prix	5	1.240		B

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
Kikuyu - Grand Prix	0.125	0.170	(-0.400, 0.650)	0.74	0.975
TifSport - Grand Prix	0.159	0.170	(-0.366, 0.684)	0.94	0.933
TifTuf - Grand Prix	0.452	0.170	(-0.073, 0.977)	2.66	0.121
Village Gree - Grand Prix	0.551	0.170	(0.026, 1.076)	3.24	0.036
Wintergreen - Grand Prix	0.150	0.170	(-0.375, 0.675)	0.88	0.947
TifSport - Kikuyu	0.034	0.170	(-0.491, 0.559)	0.20	1.000
TifTuf - Kikuyu	0.327	0.170	(-0.198, 0.852)	1.92	0.412
Village Gree - Kikuyu	0.426	0.170	(-0.099, 0.951)	2.51	0.162
Wintergreen - Kikuyu	0.025	0.170	(-0.500, 0.550)	0.14	1.000
TifTuf - TifSport	0.293	0.170	(-0.232, 0.818)	1.73	0.529
Village Gree - TifSport	0.392	0.170	(-0.133, 0.917)	2.31	0.230
Wintergreen - TifSport	-0.009	0.170	(-0.534, 0.516)	-0.06	1.000
Village Gree - TifTuf	0.099	0.170	(-0.426, 0.624)	0.58	0.991
Wintergreen - TifTuf	-0.302	0.170	(-0.827, 0.223)	-1.78	0.496
Wintergreen - Village Gree	-0.402	0.170	(-0.927, 0.124)	-2.36	0.209

Individual confidence level = 99.50%



If an interval does not contain zero, the corresponding means are significantly different.

Root Dry Weight Infested 17 weeks (log) versus Cultivar

Factor	Levels	Values
Cultivar	6	Grand Prix, Kikuyu, TifSport, TifTuf, Village Green, Wintergreen

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cultivar	5	0.5746	0.11492	3.20	0.024
Error	24	0.8612	0.03588		
Total	29	1.4358			

Grouping Information Using the Tukey Method and 95% Confidence

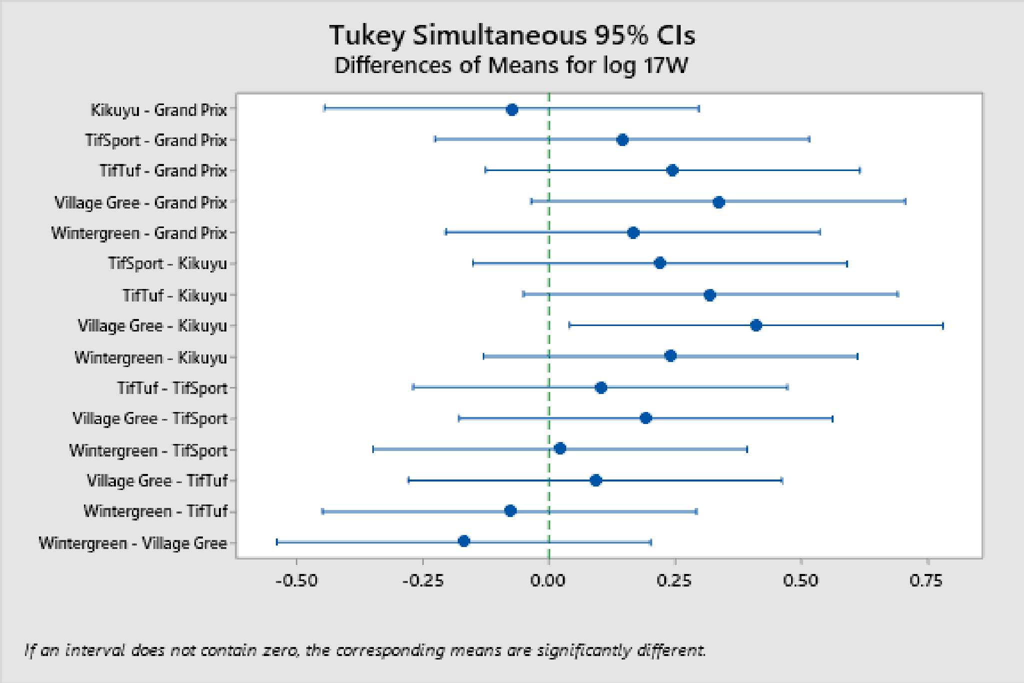
Cultivar	N	Mean	Grouping	
Village Green	5	1.5670	A	
TifTuf	5	1.477	A	B
Wintergreen	5	1.3972	A	B
TifSport	5	1.3762	A	B
Grand Prix	5	1.2327	A	B
Kikuyu	5	1.1576		B

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
Kikuyu - Grand Prix	-0.075	0.120	(-0.445, 0.295)	-0.63	0.988
TifSport - Grand Prix	0.144	0.120	(-0.227, 0.514)	1.20	0.833
TifTuf - Grand Prix	0.244	0.120	(-0.126, 0.614)	2.04	0.352
Village Gree - Grand Prix	0.334	0.120	(-0.036, 0.705)	2.79	0.094
Wintergreen - Grand Prix	0.165	0.120	(-0.206, 0.535)	1.37	0.742
TifSport - Kikuyu	0.219	0.120	(-0.152, 0.589)	1.83	0.470
TifTuf - Kikuyu	0.319	0.120	(-0.051, 0.689)	2.66	0.120
Village Gree - Kikuyu	0.409	0.120	(0.039, 0.780)	3.42	0.024
Wintergreen - Kikuyu	0.240	0.120	(-0.131, 0.610)	2.00	0.371
TifTuf - TifSport	0.100	0.120	(-0.270, 0.471)	0.84	0.957
Village Gree - TifSport	0.191	0.120	(-0.179, 0.561)	1.59	0.611
Wintergreen - TifSport	0.021	0.120	(-0.349, 0.391)	0.17	1.000
Village Gree - TifTuf	0.090	0.120	(-0.280, 0.460)	0.75	0.973
Wintergreen - TifTuf	-0.080	0.120	(-0.450, 0.291)	-0.66	0.984
Wintergreen - Village Gree	-0.170	0.120	(-0.540, 0.200)	-1.42	0.717

Individual confidence level = 99.50%



Total Plant Biomass Non-infested 8 weeks (log) versus Cultivar

Factor	Levels	Values
Cultivar	6	Grand Prix, Kikuyu, TifSport, TifTuf, Village Green, Wintergreen

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cultivar	5	0.2211	0.04423	2.69	0.046
Error	24	0.3947	0.01645		
Total	29	0.6158			

Grouping Information Using the Tukey Method and 95% Confidence

Cultivar	N	Mean	Grouping
TifTuf	5	3.2614	A
Village Green	5	3.2374	A
Grand Prix	5	3.2301	A
Wintergreen	5	3.1577	A
Kikuyu	5	3.0880	A
TifSport	5	3.0254	A

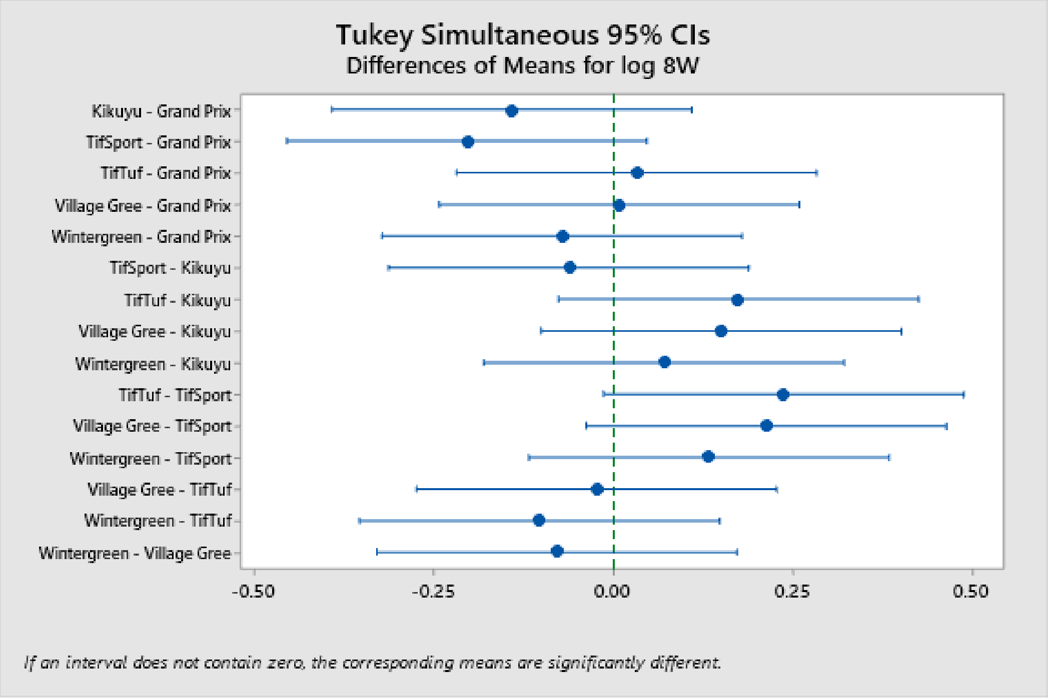
Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
Kikuyu - Grand Prix	-0.1421	0.0811	(-0.3927, 0.1086)	-1.75	0.514
TifSport - Grand Prix	-0.2047	0.0811	(-0.4553, 0.0460)	-2.52	0.157
TifTuf - Grand Prix	0.0314	0.0811	(-0.2193, 0.2820)	0.39	0.999
Village Gree - Grand Prix	0.0073	0.0811	(-0.2433, 0.2579)	0.09	1.000
Wintergreen - Grand Prix	-0.0723	0.0811	(-0.3230, 0.1783)	-0.89	0.945
TifSport - Kikuyu	-0.0626	0.0811	(-0.3133, 0.1880)	-0.77	0.970
TifTuf - Kikuyu	0.1734	0.0811	(-0.0772, 0.4241)	2.14	0.302

Village Gree - Kikuyu	0.1494	0.0811	(-0.1013, 0.4000)	1.84	0.460
Wintergreen - Kikuyu	0.0697	0.0811	(-0.1809, 0.3203)	0.86	0.952
TifTuf - TifSport	0.2360	0.0811	(-0.0146, 0.4867)	2.91	0.073
Village Gree - TifSport	0.2120	0.0811	(-0.0386, 0.4626)	2.61	0.132
Wintergreen - TifSport	0.1323	0.0811	(-0.1183, 0.3830)	1.63	0.587
Village Gree - TifTuf	-0.0241	0.0811	(-0.2747, 0.2266)	-0.30	1.000
Wintergreen - TifTuf	-0.1037	0.0811	(-0.3543, 0.1469)	-1.28	0.793
Wintergreen - Village Gree	-0.0796	0.0811	(-0.3303, 0.1710)	-0.98	0.919

Individual confidence level = 99.50%



Total Plant Biomass Non-infested 17 weeks (log) versus Cultivar

Factor	Levels	Values
Cultivar	6	Grand Prix, Kikuyu, TifSport, TifTuf, Village Green, Wintergreen

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cultivar	5	0.03684	0.007369	0.68	0.645
Error	24	0.26132	0.010889		
Total	29	0.29817			

Root Dry Weight Non-infested 8 weeks (log) versus Cultivar

Factor	Levels	Values
Cultivar	6	Grand Prix, Kikuyu, TifSport, TifTuf, Village Green, Wintergreen

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cultivar	5	0.06435	0.01287	0.47	0.795
Error	24	0.65728	0.02739		
Total	29	0.72162			

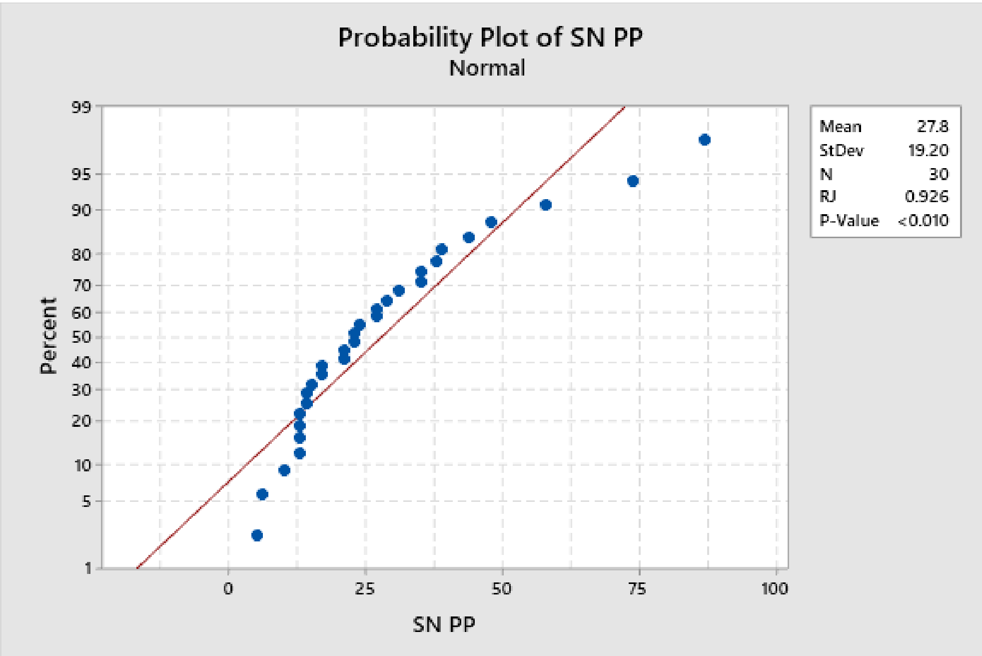
Root Dry Weight Non-infested 17 weeks (log) versus Cultivar

Factor	Levels	Values
Cultivar	6	Grand Prix, Kikuyu, TifSport, TifTuf, Village Green, Wintergreen

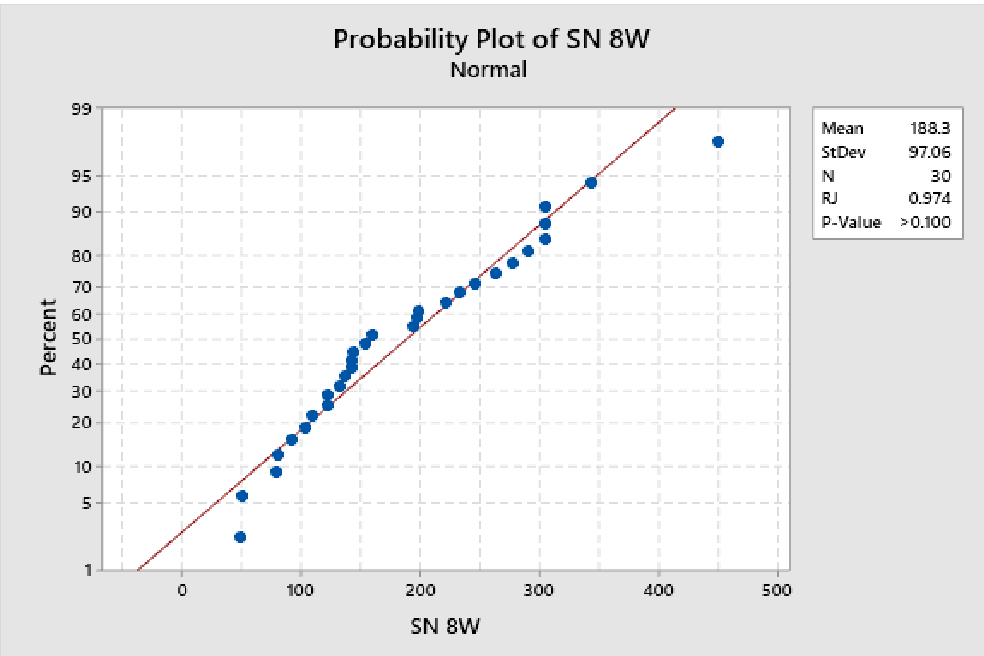
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Cultivar	5	0.02696	0.005391	0.20	0.961
Error	24	0.65890	0.027454		
Total	29	0.68585			

NORMALITY TESTS

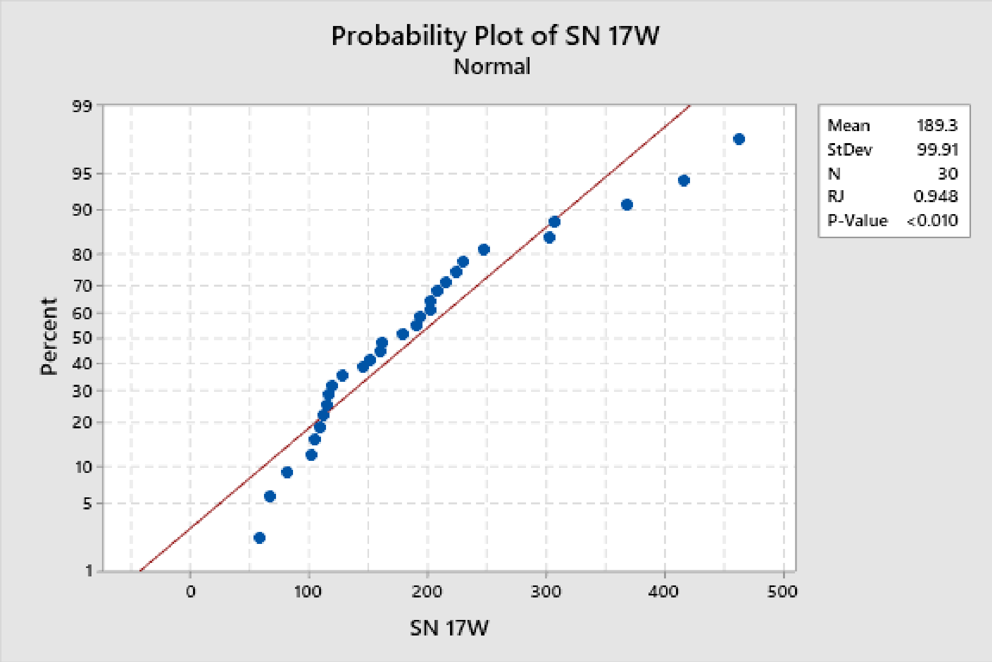
Nematode Infested Pre-plant



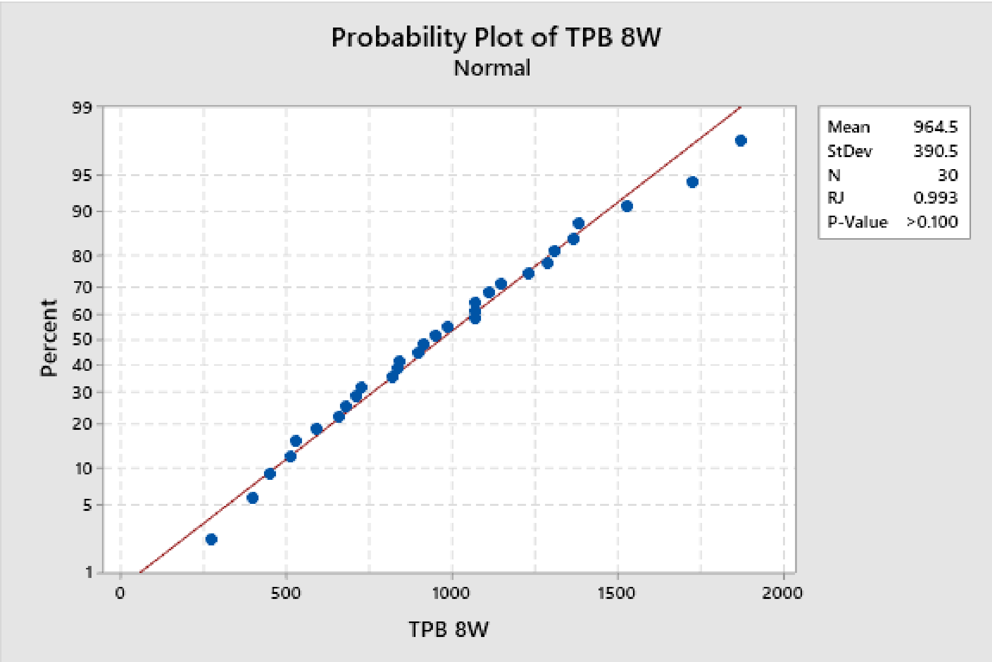
Nematode Infested 8 weeks



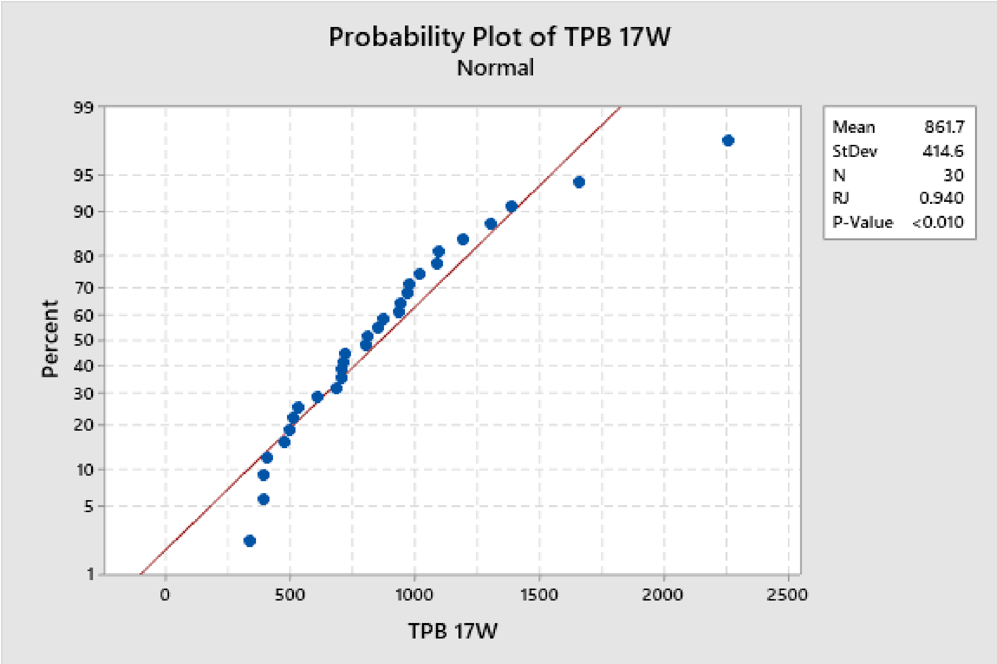
Nematode Infested 17 weeks



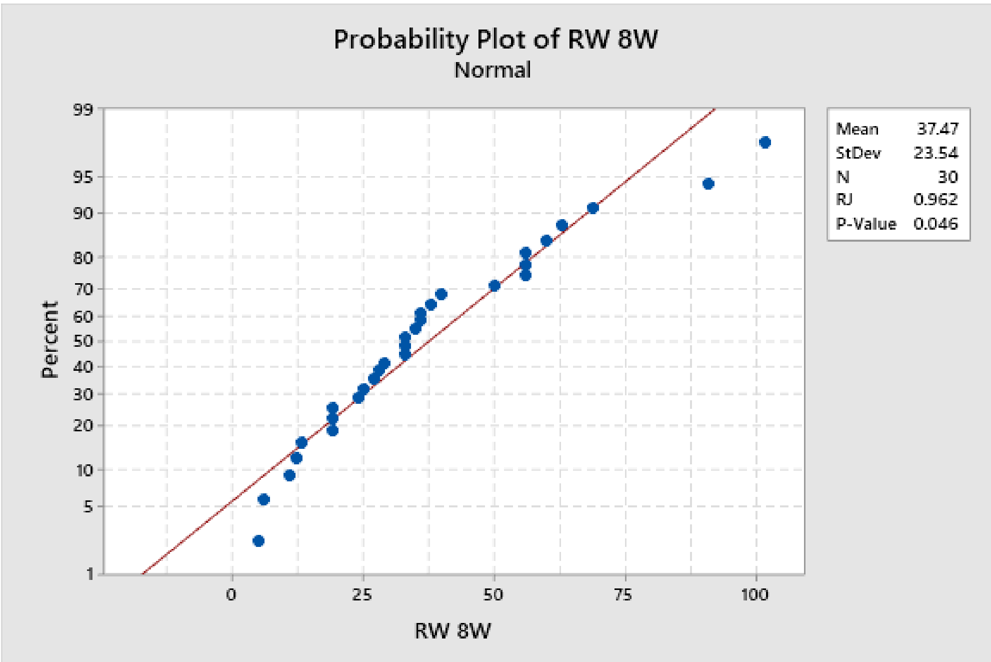
Total Plant Biomass Infested 8 weeks



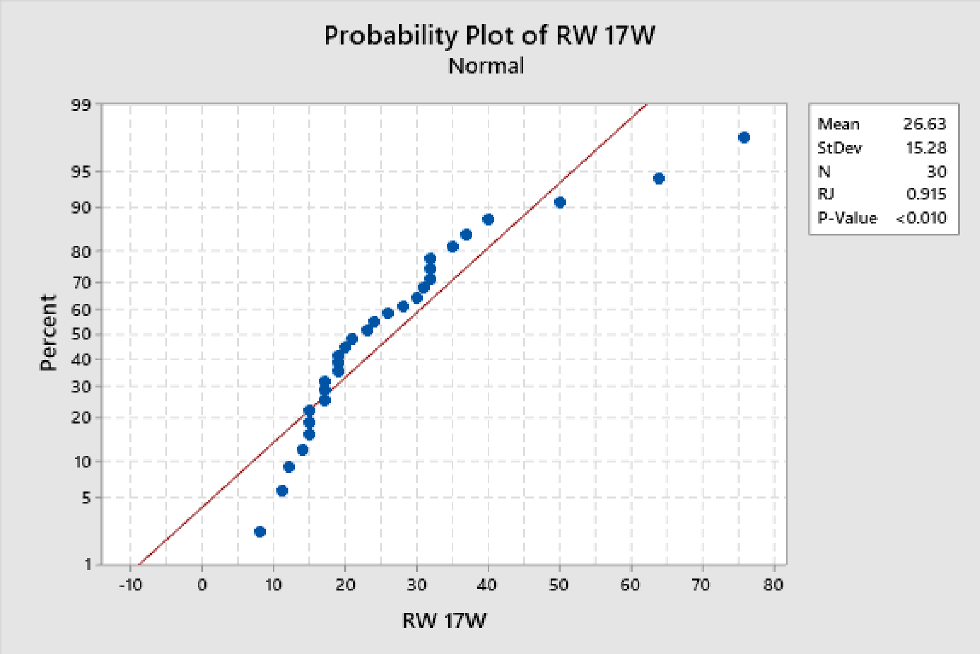
Total Plant Biomass Infested 17 weeks



Root Dry Weight (Infested) 8 weeks



Root Dry Weight (Infested) 17 weeks



TEST FOR EQUAL VARIANCES

Null hypothesis	All variances are equal
Alternative hypothesis	At least one variance is different
Significance level	α = 0.05

Nematode Infested Pre-plant

Method	Test Statistic	P-Value
Multiple comparisons	—	0.517
Levene	0.45	0.811

Nematode Infested 8 weeks

Method	Test Statistic	P-Value
Multiple comparisons	—	0.376
Levene	0.50	0.774

Nematode Infested 17 weeks

Method	Test Statistic	P-Value
Multiple comparisons	—	0.002
Levene	1.78	0.155

Total Plant Biomass Infested 8 weeks

Method	Test Statistic	P-Value

Multiple comparisons	—	0.407
Levene	0.57	0.720

Total Plant Biomass Infested 17 weeks

Method	Test Statistic	P-Value
Multiple comparisons	—	0.412
Levene	0.58	0.718

Root Dry Weight Infested 8 weeks

Method	Test Statistic	P-Value
Multiple comparisons	—	0.149
Levene	1.16	0.359

Root Dry Weight Infested 17 weeks

Method	Test Statistic	P-Value
Multiple comparisons	—	0.019
Levene	1.75	0.161

Total Plant Biomass Non-infested 8 weeks

Method	Test Statistic	P-Value
Multiple comparisons	—	0.129
Levene	1.02	0.426

Total Plant Biomass Non-infested 17 weeks

Method	Test Statistic	P-Value
Multiple comparisons	—	0.396
Levene	0.45	0.809

Root Dry Weight Non-infested 8 weeks

Method	Test Statistic	P-Value
Multiple comparisons	—	0.487
Levene	0.73	0.610

Root Dry Weight Non-infested 17 weeks

Method	Test Statistic	P-Value
Multiple comparisons	—	0.068
Levene	1.04	0.415