3736

UNIVERSITY OF HAWAI'I LIBRARY

HOT WATER DRENCH TREATMENTS FOR THE CONTROL OF BURROWING NEMATODE, *RADOPHOLUS SIMILIS,* IN TROPICAL ORNAMENTALS

A THESIS SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAII IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN

BOTANICAL SCIENCES (PLANT PATHOLOGY)

DECEMBER 2002

By

Albert C. Arcinas

Thesis Committee:

Brent Sipes, Chairperson
Arnold Hara
Donald Schmitt
Marcel Tsang

ACKNOWLEDGEMENTS

To my major advisor, Dr. Brent Sipes, and my committee members Drs. Arnold Hara, Marcel Tsang, and Donald Schmitt, thank you for your guidance and support. This project would not have been possible without the technical support of Christopher Jacobsen, Ryan Kaneko, Donna Myers, and Mike Young, and all of the students at the UH Nematology Lab. I would also like to gratefully acknowledge Big Island Plants, Hilo, HI and Cal-Hawaii Nursery, Keeau, HI, for providing the plant material used in this study.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Hot water drench treatments were investigated for their potential application as quarantine treatments against *Radopholus similis*, in two palms, *Rhapis excelsa* and *Caryota mitis*, and in potted *Anthurium*. Drenches with 50°C water were applied for 10 to16 minutes to both *R. excelsa* and *C. mitis*. *R. similis* were eliminated from *C. mitis* in all treatments longer than 10 minutes. In *R. excelsa*, a 16-minute hot water drench achieved 99.6% mortality of *R. similis*. In *Anthurium*, previous applications of hot water drench treatments resulted in a few survivors being detected 2 months after treatment. An experiment was designed to test cultivar effects, duration of time between treatment and nematode assay, and location of surviving nematodes after hot water drench treatment on four cultivars *Anthurium*. No cultivar differences were found in the reproductive factor of *R. similis*. Surviving nematodes, 1 week after treatment, were only found in stem sections above the soil line. Four weeks after treatment nematodes were found in roots and stems below the soil line. Migration into stem tissue is a proposed mechanism for escaping lethal temperatures. Conditioning treatments applied to *Anthurium* may also enhance thermotolerance in *R. similis* and decrease the efficacy of subsequent eradication treatments. *R. similis* did not survive challenge heat treatment after receiving a variety conditioning treatments *in vitro*. Probit regression estimates of conditioned and unconditioned *R. similis* mortality rates in potted *Anthurium* was similar. However survivors in conditioned potted *Anthurium* suggest that efficacy of eradication is compromised, although development of thermotolerance has not been confirmed.

CHAPTER 1

LITERATURE REVIEW

1.1. Burrowing Nematode Quarantine

As world trade increases, so does the exchange of agricultural products across borders and nations. Appropriate quarantine measures are necessary to prevent the spread of invasive plant pests through such exchange. Mutual agreements are arranged between states and nations, under the guidelines of the World Trade Organization (WTO) Application of Sanitary and Phytosanitary Measures (SPS) as to obligations and standards of quarantine that effectively exclude plant pests from entry, while avoiding unnecessary restrictions to trade (NPB, 1999). As a guiding principle these standards must be based on scientific research and judged by risk assessment (NPB, 1999). The development of effective quarantine treatments that meet national and international standards, begins with objective scientific research, avoids unnecessary and burdensome limitations to trade, and facilitates the growth of commerce and trade of agricultural commodities (NPB, 1999).

In Hawaii, an important part of the agricultural sector is the export of ornamental nursery plants. The tropical foliage and flower industry has steadily increased during the period 1995 to 1999 reaching a level of $75.4 million in sales in 1999 (Hawaii Agricultural Statistics Service, 2000). The market for these products has the potential to increase many fold since Hawaii's exports predominantly serve west coast markets of the

United States (Linney, 1990). Lack of approved quarantine treatments has been a major obstacle to export of floriculture and foliage products to Japan (GACC, 1994). Without effective quarantine treatments, Hawaii will continue with this disadvantage and market demand will be met from elsewhere.

Spreading Decline of Citrus

Radopholus similis is a quarantined pest in part because of Spreading Decline disease of citrus in Florida. Spreading Decline disease of citrus was first found in the Lucerne Park area of Polk County, Florida during 1926-28 (Holdeman, 1986). The disease is manifested as trees that are stunted and have sparse foliage. The leaves and fruit are small and entire branches may die (Luc *et al.*, 1990). This disease makes the economic production of citrus impossible. In 1953, the causal organism was identified as *R. similis* (Suit and DuCharme, 1953). Further observations found that not all isolates of *R. similis* from throughout the world parasitize citrus (Kaplan and Opperman, 1997). However, these isolates are genetically similar and do not represent distinct species, as previously proposed (Huettel, *et al.*, 1986). Variations in chromosome number, isozyme patterns, and morphology can distinguish isolates from one another, however none of these criteria are correlated with citrus parasitism (Goo and Sipes, 1999; Kaplan and Opperman, 1997). Citrus parasitism of *R. similis*, found only in Florida, is associated with limited changes in the nematodes genome and appears to be inherited as a dominant trait (Kaplan and Opperman, 1997). The differential regulation against citrus parasitic

races of *R. similis* is currently impractical due to the lack of cost effective and/or timely methods for identification (Holdeman, 1986).

Hawaii and Burrowing Nematode Quarantine

In 1955, California along with other states and nations enacted legislation to protect their respective citrus industries. An early survey in California, found 11.2 % of plants imported from Hawaii, including *Musa paradisiaca*, *Anthurium andraeanum*, *Philodendron cordatum*, *Scandapsus areus*, *Strelitzia reginae*, *Heliconia* sp., and *Hedychium coronarium*, were infested with *R. similis* (Ishii *et al.*, 1956). Broad and inclusive quarantine regulations were developed (Holdeman, 1986). The importation of all soil, plant parts with roots, and all plant cuttings for propagation from areas with known infestations of *R. similis* were restricted (CDFA, 1999). The volume of exports from Hawaii to the mainland was drastically curtailed by the initiation of quarantine laws to prohibit entry of undesired nematode species.

Pre-Shipment Certification

Each year cooperative Federal and State export programs handle roughly 270,000 shipments accounting for plant and plant products worth $23 billion (USDA-APHIS, 2001). To facilitate this volume of trade, protocols have been established by national and state regulatory agencies. In Hawaii, nurseries desiring to export plants are certified through a program with the Hawaii Department of Agriculture (HDOA), authorized by the national Plant Protection Act of 2000, and under the auspices of the USDA Animal and Plant Health Inspection Service, Plant Protection and Quarantine (USDA-APHIS-

PPQ) (HDOA, 1981). The PPQ staff, in cooperation with state, county officials, and industry members, is charged with facilitation of exportation of plants and plant products. For interstate shipments, exporters must obtain clearance through a preshipment certification program or must submit to phytosanitary inspection and certification of each shipment. A preshipment certification program expedites transfer of plants by requiring the grower to meet specific guidelines of production prior to shipment (USDA-APHIS, 2001). For instance, Burrowing Nematode (BN) certification of potted citrus stock in Florida requires the grower to raise benches 48-cm above the ground and mandates numerous samplings of the growing stock each year (FDACS, 2001). There are similar production specifications for export certification in Hawaii. A nursery's certification may be revoked upon receipt of an out-of state rejection notice for a quarantined pest (HDOA, 1981). International trade follows a similar procedure. For US exports, APHIS-PPQ provides phytosanitary inspections and documentation that meet specific quarantine protocols for each country and commodity. When shipments are infested with a disease or insect, the shipment is returned to the country of origin, held at port until the products are treated with approved quarantine treatments, or destroyed at the grower's expense. Since valuable shipments are transported in sea cargo containers and are rejected *in toto* interception of quarantined pests occurs at a substantial loss to growers.

Therapeutic Options

Since there are no nematicides available for therapeutic eradication of plant-parasitic nematodes in ornamentals, growers find themselves in a predicament whenever

nematode infestations occur in plants destined for export. With no available treatment, growers must destroy valuable plants, relegate infected plant material to non-quarantine markets, or rely upon preventive measures, and begin the costly process of locating and eliminating the source of contamination (Ishii *et al.*, 1956; Holtzmann *et al.*, 1984). The overall goal of this thesis is to develop an effective therapeutic treatment to eliminate plant-parasitic nematodes that meets quarantine standards.

1.2 *Radopholus similis*, The Burrowing Nematode

Radopholus similis (Cobb, 1893) is a roundworm about 0.65 mm long by 25 μm wide (Thorne, 1961). The nematode spends most of its life inside cavities in the root cortex, where it completes a life cycle in about 25-30 days (Luc *et al.*, 1990). All juveniles and females can infect roots, emerge from the roots, and spread through the soil (Holdeman, 1986). The nematode penetrates anywhere along the root, and enters parenchyma layer beneath the epidermal cells. From this intracellular location, the nematode inserts its stylet through the cell wall, and feeds directly on the cytoplasm of the cell. The drained cell eventually collapses, and a cavity is formed. As feeding continues the cavities coalesce and resemble tunnels or lesions on the surface of the root (Thorne, 1961). The nematode is unable to penetrate the suberized endodermis surrounding the vascular tissue (Holdeman, 1986). However, secondary fungal invaders are early colonizers of lesions formed by *R. similis*. A pathogenic synergism can occur between *R. similis* and secondary invaders, as subsequent pathogens are unable to colonize roots of *Musa acuminata* 'Dwarf Cavendish', unless first penetrated by *R.*

similis (Blake, 1966). The mycelia of fungi such as *F. oxysporum* are then able to penetrate the suberized endodermis and damage vital vascular tissue, weakening the taproot (Blake, 1966). The physiological result is the loss of anchoring and may lead to toppling of banana laden trees. High nematode populations and the resulting death of tissue force nematodes to migrate through the soil in search of new feeding sites. Most plant to plant spread is through root contact or near contact facilitated by water (Holdeman, 1986). Long-distance spread is primarily through movement of infected plant material or infested soil (Luc *et al.*, 1997).

In Hawaii, *R. similis* is a major pest of *A. andraeanum, M. paradisiaca*, and tropical foliage such as, *Chamaedorea seifrizii*. Infection can cause significant reduction in flower or fruit yield (Aragaki *et al.*, 1984; Araya *et al.*, 1999). In tropical palms, the most conspicuous symptoms of infestation are lesions and the rotting of roots, which cause overall yellowing of the plant and slower growth (Luc *et al.*, 1990).

1.3 Approved Quarantine Heat Treatments

Heat treatments have been used to control plant diseases and insects for many years. Heat treatments may be applied to agricultural commodities: (i) by immersion in hot water, (ii) exposure to vapor heat, (iii) exposure to hot dry air, (iv) treatment with infrared radiation, or (v) by microwave radiation. The most practical treatments with respect to costs, minimization of damage, and efficacy have been vapor heat, hot air, and hot water (Tsang *et al.*, 1995; Sharp *et al.*, 1990; Gaffney and Armstrong, 1990).

Vapor Heat

Vapor heat uses warm air saturated with water vapor at temperatures between 40 and 50°C (Gaffney *et al.*, 1990). Vapor heat was first used on a large scale as a quarantine treatment for the Mediterranean fruit fly in Florida in the early 1930's (Couey, 1989). Vapor heat has been effective for many tropical fruits and vegetables, including the disinfestation of papayas from *Dacus dorsalus* and *D. cucurbitae* in Hawaii (Couey, 1989; NOSB, 1998; U.S. EPA, 1996; Gaffney *et al.*, 1990). Vapor heat has been approved by PPQ for treatment of papayas, mangos, and pineapple mainly for the control of fruit flies. However, vapor heat quarantine treatments occasionally produce scald and shriveling of mangoes and pitting in papayas (Sharp *et al.*, 1991; Gaffney and Armstrong, 1990).

Hot Air

Hot air treatments of 40 to 50°C can lessen damage to fruit by preventing condensation of moisture on the fruit surface (Armstrong *et al.*, 1989). Damage is abated by keeping the dew point temperature of the air 2 to3°C below the fruit surface temperature. The dew point temperature must be increased as fruit surface temperature increases to avoid fruit desiccation (Sharp *et al.*, 1991; Gaffney and Armstrong, 1990). Precise computer control of recirculated heated air and humidity are necessary to limit the rate of fruit heating. For papayas, a heating method at four incrementally increased temperatures between 40 and 50°C over an 8-hour period was developed (Armstrong *et al.*, 1989). Other fruits that tolerate hot air treatments for the control of *Tephritidae* pests

are mango (Mangan and Ingle, 1992), grapefruit (Sharp and Gould, 1994), navel orange (Sharp and McGuire, 1996), carambola (Sharp and Hallman, 1992), and persimmon (Lay-Yee, 1994). Avocado, lychee, and nectarine are damaged at temperatures not lethal to the targeted pests (Sharp 1994; Kerbel *et al.*, 1987). Hot air treatments for grapefruit, papaya, and mango are approved by USDA-APHIS as quarantine treatments against various *Tephritidae* species (APHIS, 1993).

Hot Water Immersion

Hot-water immersion treatments also have quarantine utility. By submerging the commodity in a hot-water bath at a constant temperature for a specified time, consistent with the thermal death point of the targeted pests yet within the thermotolerance of the commodity, both disinfestation and product quality can be achieved. The rapid heat transfer of water allows large amounts of material to reach uniform temperature when submerged (U.S. EPA, 1996). Temperature-duration combinations vary for different commodities, targeted pests, and life stages of insects. In general, temperature must reach 43-47°C with exposure times ranging from 35 to 90 minutes to control various *Tephritidae* species (APHIS, 1993). Hot-water immersion treatment is a USDA-APHIS approved quarantine treatment for limes imported from Chile, all mangos, and several less economically important tropical fruits (U.S. EPA, 1996). Tropical floral commodities such as *Strelitzia reginae, Gardenia jasminoides, Alpinia purpurata, Heliconia* sp., have been disinfested of aphids, soft scales, armored scales, mealybugs, thrips, and other surface insect pests without phytotoxic damage by immersion in hot

water for 6 to 12 minutes at 49 °C (Hara *et al.*, 1994; Hara *et al.*, 1994; Tsang *et al.*, 1995). In addition, hot-water immersion has the additional benefit of controlling postharvest microbial diseases such as anthracnose and stem end rot (Couey 1989; McGuire, 1991). Currently, no commercial system procedure is approved by USDA-APHIS for tropical flowers and cut foliage. The only USDA-APHIS quarantine treatments approved for cut flowers are hand removal, chemical dips, and methyl bromide fumigation (Tsang *et al.*, 1995).

In order for quarantine treatment to be approved by PPQ, the efficacy of treatment must meet or exceed the USDA probit 9 security level standard of 99.9968% mortality at the 95% confidence level (maximum of 32 survivors in a million treated individuals) (U.S. EPA, 1996). This high level of mortality was initially recommended for fruit flies in heavily infested commodities to prevent a potential mating pair from surviving a shipment of fruit (Follett, 1999).

1.4. Hot Water Immersion and Nematodes

Plant-parasitic nematodes have been controlled by hot water immersion. *Meloidogyne incognita* and *Helicotylenchus multicinctus* were killed in vitro with a 4-minute exposure to 50°C (Birchfield and van Pelt, 1958). In many bareroot ornamentals tests for nematode presence and thermotolerance at 50°C for a 10-minute duration, a high variability in the survival of nematodes among the plants tested was found. Seven unrelated plant species caused gall formation on a bioassay plant, such as the woody

perennial *Buxus sempervirens* and the tuberous tropical *Caladium bicolor* (Birchfield and van Pelt, 1958).

Heat Tolerance of Plants

In general, heat tolerance is a function of size and woodiness of roots. Succulents and herbaceous plants were generally damaged most by the immersion in hot water. However, *Gardenia jasminoides* cv. 'Ellis' and *B. sempervirens*, both woody perennials, suffered high mortality (Birchfield and van Pelt, 1958). Tolerance and efficacy of hot water treatments seems to be plant species specific and must be examined on a case-by-case basis.

Treatment of *M. incognita* and *Pratylenchus vulnus* in grapevine rootings by hot water immersion has also been investigated (Lear and Lider, 1959). A hot water treatment of cuttings was 100% effective against *M. incognita* at temperatures as low as 48°C for 30 minutes (Lear and Lider, 1959). Higher temperatures allowed shorter treatment durations without sacrificing efficacy. Optimal duration and temperature with high efficacy was achieved at 50°C for 10 minutes. No injury to either shoots or roots was observed at temperatures below 54°C in grape rootings (Lear and Lider, 1959). Similar results were obtained for *P. vulnus*. Hot water treatment of grapevine cuttings is approved by the California Department of Food and Agriculture (CDFA) for certification of *M. incognita* free stocks (Lear, 1966).

Integrated Hot Water Treatments with Chemical and Cultural Controls

Hot water treatments as a stand-alone treatment or in combination with safer alternatives to aqueous formaldehyde were investigated for the control of stem and bulb nematode, *Ditylenchus dipsaci* (Roberts and Matthews, 1995). Long-term exposure to formaldehyde is carcinogenic and short-term exposure to the gas of formaldehyde can be fatal (OSHA, 2001). Complete control of *D. dipsaci* with only hot water could not be achieved without retarding early plant emergence, although normal plant development occurred at a later stage (Roberts and Matthews, 1995). The upper limit of heat treatments was limited by the low heat tolerance of *Allium sativum* seed cloves, which are readily injured by a few minutes exposure to temperatures above 49°C (Lear and Johnson, 1962). No significant improvement in control was observed with hot water dip times of 15-30 minutes at 49°C, preceded by a conditioning dip of 30-minutes at 38°C, over the hot water-formalin dip. However, nematode mortality increased with the longer dips when compared to the controls (Roberts and Matthews, 1995).

Similar investigations into hot water were undertaken on *Narcissus pseudonarcissus* bulbs when chemical treatments such as 1,3-dichloropropene and fenamiphos lost their registration (Qiu *et al.*, 1993). In this study, hot water efficacy on *D. dipsaci* was inversely related to treatment temperature. At 150, 60, 15, and 5 minutes, 100% mortality was achieved at 44, 46, 48, and 50°C respectively. The treatment temperature was measured in the internal tissue of the bulb. The bulb circumference was found to have a uniform linear relationship with the time required for 100% efficacy at 44, 48, or 50°C (Qiu *et al.*, 1993). Qiu determined that in order for hot water treatments

to have dependable efficacy, bulbs would have to be sorted by size. Hot water as a stand-alone treatment had satisfactory efficacy at 44 °C for 240 minutes (Qiu *et al.*, 1993).

Integrated hot water, chemical, and cultural controls of *R. similis* were investigated in *M. acuminata* 'Dwarf Cavendish' propagative rhizomes. Hot water at 55°C for 20 minutes, the chemical Phoshamidon, the bio-agent Neem, and a cultural control of paring (removing outer skin of propagative rhizomes until white portion is exposed) were compared individually and in combination for efficacy (Ravichandra and Krishnappa, 1985). Maximum control was recorded for two combinations, hot water in combination with paring, and Neem in combination with paring and Phosphamidon. Among all the treatments, hot water alone recorded the maximum plant height, girth of pseudostem, and minimum root lesion index (Ravichandra and Krishnappa, 1985). In Hawaii, several varieties of banana have been disinfested of nematodes after 10-minutes at 50°C after paring as an established commercial treatment of propagative rhizomes to avoid introducing infested plants into new fields (Trujillo, 1964).

1.5. Hot Water Immersion on Tropical Ornamentals

Preliminary work with nematodes on tropical ornamentals with hot water drenching unit has been initiated (Tsang *et al.*, 2001). A hot water drenching system consisting of a hot water reservoir, water circulation and delivery system, temperature control and monitoring unit has been developed and tested (Tsang *et al.*, 2001). A comparison study was made on the efficacy of hot water in bareroot plants dipped, potted plants drenched, or potted plants dipped. Complete control of nematodes was achieved in

both bare root plants dipped and potted plants drenched (Tsang *et al.*, 2001). Nematodes were recovered from the potted plants that were dipped. Temperature probes indicated that the media surrounding the dipped potted plants never reached treatment temperature of 50°C after 15 minutes and may be the reason for nematode survival (Tsang *et al.*, 2001).

Potted *Chamaedorea seifrizii*

Potted *Chamaedorea seifrizii* inoculated with *R. similis* were drenched with hot water at 50°C for 15 and 20 minutes. Plants from both treatments were free of nematodes. Only minimal heat injury occurred to the plants, exhibited by leaf senescence of the bottom outer leaves that had been in contact with the hot water. No further phytotoxicity was recorded after these leaves were removed (Tsang *et al.*, 1999).

Potted *Anthurium andraeanum*

Potted *A. andraeanum* plants drenched with 49°C water for 10 or more minutes were free of nematodes. However, upon mist extraction of roots 2-months later, some plants had low numbers of nematodes in roots or stems (Sipes *et al.*, unpublished). Whether this was due to survival through treatment or through reinfestation from the environment during the observation period is unknown. *R. similis* is a migratory endoparasitic nematode and has been detected in the stem tissue of *A. andraeanum* (Wang and Sipes, 1999). Any nematodes that are in the stem tissue will not be affected by hot water drenches of roots. A long observation period of 2-months between treatment and extraction of nematodes may have allowed migratory nematodes to reinfest

treated and disinfested roots. Cultivars will vary upon their resistance or tolerance to *R. similis* infestation (Wang *et al.*, 1998). Some commercial cultivars were developed as hybrids crossed from 3 to 4 different species with the primary objective to develop specific horticultural attributes, such as flower size, color, and growth habits, so any differences vis á vis nematode resistance are purely incidental (Kamemoto & Kuehnle, 1996).

Currently, plant quarantine sampling for phytosanitary certification is mist extraction of root samples and will not detect residual nematodes in the stem (Kashiwamura, HDOA, personal communication). The location of any surviving nematodes in the root system must be established to determine whether hot water treatments can provide 100% efficacy against *R. similis*. This will determine whether further modifications of hot water treatment will be necessary to eradicate nematodes in the stem.

These results have established hot water treatments as potential methods for controlling plant-parasitic nematodes. However, each plant-nematode combination has its own temperature-time requirements and must be assessed individually.

1.6. Heat Conditioning to Increase Heat Tolerance

Tropical floral and foliage commodities including *A. andraeanum*, *Leucospermum* sp., *Alpinia purpurata*, and *Arundina graminifolia*, are sensitive to heat treatments (Hansen et al, 1992; Ishii, 1956). However, a conditioning heat treatment has been observed to overcome damaging effects of subsequent heat treatments in several

agricultural commodities including *Phaseolus vulgaris, Vigna sinensis, Zea mays, Cucumis sativus, Ficus carica, Glycine max, Helianthus annuus,* and *Nicotiana tabacum* (Yarwood, 1967). Primary leaves of *V. sinensis* exhibited maximum temperature adaptation when conditioned with 20 seconds of 50°C hot water followed by 8 hours at 40°C in an air incubator. Lag periods of around 3 hours were required between conditioning treatment and challenge heat (Yarwood, 1967).

Heat Shock Proteins

Increased thermotolerance is associated with heat shock proteins (HSP) that are synthesized when temperatures rise 5 to 10°C above ambient but are optimally induced at 37 to 40°C in hot air over a 2-hour exposure (Paull and Chen, 1990). Higher conditioning temperatures may allow shorter treatment periods. The presence of these novel proteins is correlated with tolerance of otherwise impermissible temperatures and the decay of HSP also corresponds to loss of thermotolerance (Paull and Chen, 1990). Increased thermotolerance is also associated with field-grown cotton (Burke *et al.*, 1985), papayas (Paull and Chen, 1990), and cucumbers (Chan and Linse, 1989) during postharvest treatments. Studies with *A. purpurata* demonstrated that conditioning with hot air at 39°C, 62% r.h., for 2 hours prior to hot-water immersion at 49°C for 12-minutes, increased flower vase life when compared to untreated controls (Hara *et al,* 1997; Paull and Chantrachit, 1998). Conditioning treatments of *Allium sativum* for the stem nematode, *Ditylenchus dipsaci*, showed extending conditioning beyond 30 minutes

did not increase treatment efficacy nor did it induce heat tolerance of *D. dipsaci* (Roberts and Matthews, 1995).

D. dipsaci nematodes exhibit induced thermotolerance when stored at 30°C for 3 to 7 days (Green, 1964). These nematodes developed near complete resistance to a hot water treatment of 46°C for 2 hours. For practical purposes of quarantine treatments, it is of concern whether conditioning plants for thermotolerance will also condition nematodes to hot water treatments. Results are inconclusive because conditioning treatments of *D. dipsaci* are meant to reproduce warm storage conditions of bulbs, which are used commercially, as they increase field germination and increase tolerance to subsequent heat treatments (Slootweg, 1962). Warm storage typically lasts one week, a conditioning period much longer than those applied to plants, which range from a few minutes to a few hours (Yarwood, 1967).

1.7. Objectives of Research

Hypothesis of Research

Hot water drenching can be an effective quarantine treatment for disinfecting roots and media of potted tropical flowers and foliage of plant-parasitic nematodes. The efficacy of this hot water treatment will meet or exceed the USDA probit 9 security level standard for efficacy.

Optimal Hot Water Drench Treatments for *Rhapis excelsa* and *Caryota mitis*

Plant species exhibit a wide range of tolerance for heat treatments from highly susceptible to extremely tolerant. However, nematodes are disinfested from potted media

with a minimum of 48°C. It is imperative to test a range of temperature and time combinations for each plant species so than heat damage is minimized while heat treatment efficacy is maximized. Two commercially important palms will be tested to develop optimal duration and temperature treatments.

Location of Surviving Nematodes in *Anthurium*

Hot water drenches of potted *Anthurium* present a challenge because survivors were detected in plants 2-months after treatment. Sporadic escapes could be due to reinfestation from surrounding environment during observation period, correlated with highly susceptible *Anthurium* cultivars, or to the ability of nematodes to migrate into stem tissue that is not directly exposed to hot water and thus does not reach target temperature. These variables must be tested in order achieve complete of control of *R. similis* infestations in potted *Anthurium*.

Effects of Conditioning Treatments for *Anthurium* on Thermotolerance of *Radopholus similis*

Conditioning plants to heat treatments are effective means for raising thermotolerance of plants and improving the efficacy of subsequent heat treatments. However, some species of nematodes have been documented to likewise improve thermotolerance when subjected to elevated non-lethal temperatures. It is of interest, whether conditioning infested plants may inadvertently condition nematodes thus reducing the efficacy of subsequent heat treatments. One objective is to subject

nematodes to the same conditioning treatments that plants receive and then test whether efficacy of hot water treatments are compromised by such conditioning.

1.8. Literature Cited

APHIS. 1993. Plant Protection and Quarantine Treatment Manual. United States Department of Agriculture. Animal and Plant Health Inspection Service.

Aragaki, M. W., J. Apt, R. K. Kunimoto, W.H. Ko and J.Y. Uchida. 1984. Nature and control of anthurium decline. *Plant Disease*. 68:509-511.

Araya, M., A. Vargas, and A. Cheves. 1999. Nematode distribution in roots of banana (*Musa* AAA cv. Valery) in relation to plant height, distance to pseudostem and soil depth. *Nematology*. 1:711-716.

Armstrong, J.W., J.D. Hansen, B.K. Hu, and S.A. Brown. 1989. High-temperature, forced-air quarantine treatment for papayas infested with Tephritid fruit flies (*Diptera: Tephritidae*). *Journal of Economic Entomology*. 82:1667-1674.

Barker, K.R. 1985. Sampling Nematode Communities. *An Advanced Treatise on Meloidogyne Vol II Methodology*. Barker, K.R., Carter, C.C., Sasser J.N. (eds). North Carolina State University Graphics: Raleigh, N.C. p.25-28.

Birchfield W. and H.M. van Pelt. 1958. Thermotherapy for nematodes of ornamental plants. *Plant Disease Reporter*. 42:451-455.

Blake, C.D. 1966. The histological changes in banana roots caused by *Radopholus similis* and *Helicotylenchus multicinctus*. *Nematologica*. 12:160-162.

Burke, J.J., J.L. Hatfield, R.R. Klein, and J.E. Mullet. 1985. Accumulation of heat shock proteins in field-grown cotton. *Plant Physiology*. 78:394-398.

Byrd D.W., Jr, Barker K.R., Ferris, H., Nusbaum, C.J., Griffin W.E., Small, R.H., Stone, C.A.. 1976. Two semi-automatic elutriators for extracting nematode and certain fungi from soil. *Journal of Nematology*. 8:206-212.

California Dept of Food and Agriculture (CDFA). Plant Quarantine Regulations 1996-7 revised 1999. CDFA. Available on the web at: www. cdfa.ca.gov/plant/pe/regs.htm

Chan, H.T., Jr. and E. Linse. 1989. Conditioning cucumbers for quarantine heat treatments. *HortScience*. 24:985-989.

Couey, H.M. 1989. Heat treatment for control of postharvest diseases and inspect pests of fruits. *Hort Science*. 24:198-202.

GACC (Governor's Agriculture Coordinating Committee). Cut Flower Industry Analysis Aug 31, 1994. Halloran, J.M., N.P Kefford, K.G. Rorbach, and L.M. Lebeck, (eds). CTAHR. University of Hawaii.

Finney, D.J. 1971. *Probit Analysis.* 3rd Edition. Cambridge University Press: Cambridge, UK.

Florida Dept. of Ag and Consumer Services (FDOAC). 2001. Rules of the FDOAC, Div. Of Plant Industry, Chapter 5B-44, Nematodes of Citrus. FDOAC. Available on-line at: http://doacs.state.fl.us/~pi/5b-44.htm

Follett, P. A., and G. T. McQuate. 2001. Accelerated quarantine treatment development for insects on poor hosts. *Journal of Economic Entomology*.. 94(5): (Forum section)

Gaffney, J.J., G.J. Hallman, and J.L. Sharp. 1990. Vapor heat research unit for insect quarantine treatments. *Journal of Economic Entomology*. 83:1965-1971.

Gaffney, J.J. and J.W. Armstrong. 1990. High-temperature forced-air research facility for heating fruits for insect quarantine treatments. *Journal of Economic Entomology.* 83:1959-1964.

Goo, M.Y.C. and B.S. Sipes. 1999. Chromosome number and reproductive isolates of *Radopholus similis* from Hawaii. *International Journal of Nematology.* 9:43-46.

Goo, M.Y.C. and B.S. Sipes. 1997. Host preference of *Radopholus citrophilus* from hawaiian anthurium among selected tropical ornamentals. *HortScience.* 32:1237-1238.

Green, C.D. 1964. The effects of high temperatures on aqueous suspensions of stem eelworm, *Ditylenchus dipsaci* (Kühn) Filipjev. *Annals of Applied Biology.* 54:381-390.

Hansen, J.D., A.H. Hara, and V.L. Tenbrink. Vapor heat: A potential treatment to disinfest tropical cut flowers and foliage. *HortScience.* 27:139-143.

Hara, A.H., T.Y. Hata, B.K.S. Hu, R.T. Kaneko, and V.L. Tenbrink. 1994. Hot water immersion of cape jasmine cuttings for disinfestation of green scale (*Homoptera: Coccidae). Journal of Economic Entomology.* 87:1569-1573.

Hara, A.H., T.Y. Hata, B.K.S. Hu, and V.L. Tenbrink. 1993. Hot water immersion as a potential quarantine treatment against *Pseudaulapsis cockerelli (Homoptera: Diaspididae). Journal of Economic Entomology.* 86:1167-1170.

Hara, A.H., T.Y. Hata, B.K.S. Hu, and M.M.C. Tsang. 1997. Hot-air induced thermotolerance of red ginger flowers and mealybugs to postharvest hot-water immersion. *Postharvest Biology and Technology.* 12:101-108.

HDOA (Hawaii Dept of Agriculture). 1981. Title 4 Subtitle 6 Chapter 73 Plant and Non-Domestic Animal Quarantine Plant Export Rules. State of Hawaii, Board of Ag.

Holdeman, Q.L. 1986. The Burrowing Nematode, *Radopholus similis, sensu lato.* California Dept. of Food and Agr., Div. of Plant Industry, Sacramento.

Holdeman, Q.L. 1986. The burrowing nematode, The citrus pathotype. California Department of Food and Agriculture, Division of Plant Industry, Sacramento.

Holtzmann, O.V., A.P. Martinez, W.J. Apt. 1984. Burrowing Nematodes: A menace to Hawaii nurseries. Honolulu: Hawaii Inst. of Trop Agriculture and Human Res. Univ. of Hawaii Info. Text Series. 20.

Huettel, R.N., Kaplan, D.T., Dickson, D.W. 1986. Characterization of a new Burrowing Nematode population, *Radopholus citrophilus,* from Hawaii. *Journal of Nematology.* 18:50-54.

Ishii, M., H. Kamemoto, T.K. Maeda, R.K.T. Au. 1956. Investigations on the control and distribution of the Burrowing Nematode. Final Report to Hawaii Economic Planning and Coordination Authority. Grant no. 5. State of Hawaii.

Kamemoto, H. & A.R. Kuehnle, 1996. Breeding *Anthurium* in Hawaii. University of Hawaii Press, Honolulu. 132 pp.

Kaplan, D.T. and C.H. Opperman. 1997. Genome similarity implies that citrus-parasitic burrowing nematodes do not represent a unique species. *Journal of Nematology.* 29:430-440.

Kerbel, E.L., F.G. Mitchell, and G. Mayer. 1987. Effect of postharvest heat treatments for insect control of the quality and market life of avocados. *HortScience.* 22:92-94.

Key, J.L., Kimpel J., Vierling, E., Lin C.Y., Nagao, R.T., Czarnecka, E., Schöffl. 1985. Physiological and molecular analyses of heat shock response in plants. *Changes in Eukaryotic Gene Expression in Response to Environmental Stress.* Atkinson, B.G. and D.B. Walden (eds). Academic Press: New York. pp 327-348.

Klein J.D. and Lurie, S. 1992. Heat Treatments for Improved Postharvest Quality of Horticultural Crops. *HortTechnology.* 2:316-320.

Ko, M.P. D.P. Schmitt, and B.S. Sipes. 1996. Axenizing and culturing endomigratory plant-parasitic nematodes using Pluronic F127, including its effects on population dynamics of *Pratylenchus penetrans. Journal of Nematology.* 28:115-123.

Lear, B. 1966. Hot-water treatment of grapevine rootings for eradication of root-lesion nematode, *Pratylenchus vulnus. Plant Disease Reporter* 50:858-859.

Lear, B. and L.A. Lider. 1959. Eradication of root-knot nematodes from grapevine rootings by hot water. *Plant Disease Reporter* 43:314-317.

Linney, P. 1990. Wholesaling Floral Products from Hawaii to the Midwest and East Coast. Proceedings of the Hawaii Tropical Cut Flower Industry Conference. CTAHR. University of Hawaii.

Luc, M., R.A. Sikora, and J. Bridge (eds). *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture.* CAB International Inst. of Parasitology: Wallingford, UK., 1990. pp. 372-373, 380-381.

Mangan, R.L. and S.J. Ingle. 1992. Forced hot-air quarantine treatment for mangoes infested with West Indian fruit fly (*Diptera: Tephritidae*). *Journal of Economic Entomology*. 85:1859-1864.

McGuire, R.G. 1991. Concomitant decay reductions when mangoes are treated with heat to control infestation of Caribbean fruit flies. *Plant Disease*. 75:946-949.

National Plant Board (NPB). 1999. Safeguarding American Plant Resources: A Stakeholder Review of the APHIS-PPQ Safeguarding System. Pest Exclusion Committee of NPB Report. Available on-line at: http://www.aphis.usda.gov/ppq/safeguarding/

NOSB (National Organic Standards Board). 1998. Proposed Recommendations on Fumigation. NOSB Issue Paper and Proposed Recommendations Oct. 27, 1998. USDA. Available on the web at: www.ams.usda.gov/nop/NOSB.

Occupational Safety and Health Administration. Mar. 16, 2001. Formaldehyde. Available on line at: http//: www.osha-slc.gov/nop/NOSB.

Paull, R.E., and Chen, N.J. 1990. Heat shock response in field-grown, ripening papaya fruit. *Journal of American Society of Horticultural Science*. 115:623-631.

Paull R.E., and Chantrachit, T. 1998. Effect of hot water on red ginger (*Alpinia purpurata)* inflorescence vase life. *Postharvest Biology and Technology*. 14:77-86.

Qiu, J., B.B. Westerdahl, D. Giraud, and C.A. Anderson. 1993. Evaluation of hot water treatments for management of *Diteylenchus dipsaci* and fungi in daffodil bulbs. *Journal of Nematology*. 25:686-694.

Rachichandra, N.G. and K. Krishnappa. 1985. Effect of various treatments, both individually and in integration, in controlling the burrowing nematode, *Radopholus similis,* infesting banana. *Indian Journal of Nematology*. 15:62-65.

Roberts, P.A. and W.C. Matthews. 1995. Disinfection alternatives for control of *Ditylenchus dipsaci* in garlic seed cloves. *Journal of Nematology*. 27:448-456.

Sharp, J.L., 1994. Hot-air-alternative quarantine treatment for methyl bromide fumigation to disinfest fruits. In: Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions. November 13-16, 1994. pp. 65-1 65-6

Sharp J.L., J.J. Gaffney, J.I. Moss, and W.P. Gould. 1990. Hot-air treatment device for quarantine research. *Journal of Economic Entomology*. 84:520-527.

Sharp, J.L. and W.P. Gould. 1994. Control of Caribbean fruit fly (*Diptera: Tephritidae*) in grapefruit by forced hot air and hydrocooling. *Journal of Economic Entomology.* 87:131-133.

Sharp, J.L. and G.J. Hallman. 1992. Hot-air treatment for carambolas infested with Caribbean fruit fly (*Diptera: Tephritidae*). *Journal of Economic Entomology.* 85:168-171.

Sharp, J.L. and R.G. McGuire. 1996. Control of Caribbean fruit fly *Diptera: Tephritidae*) in navel orange by forced hot air. *Journal of Economic Entomology.* 89:1181-1185.

Slootweg, A.F.G. 1962. Hot water treatment of daffodils. *Daffodil Tulip Yearbook.* 1963:82-87.

Suit, R.F. and E.P. DuCharme. 1953. The burrowing nematode and other parasitic nematodes in relation to spreading decline of citrus. *Plant Disease Reporter*. 37:379-383.

Hawaii Agricultural Statistics Service: Hawaii Flowers & Nursery Products. 2000. Hawaii Department of Agriculture. P.O. Box 22159, Honolulu, HI 96823-2159. Available on-line at: http://www.nass.usda.gov/hi/flower/foliag99.htm.

Thorne, G. 1961. *Principles of Nematology*. McGraw-Hill: New York, pp. 226-232.

Trujillo, E.E. 1964. Clean banana rhizome certification. *Hawaii Farm Science*. 14(Oct): 8-9.

Tsang, M.M.C., Hara, A.H., Sipes B.S. 2001. Hot Water Drenching System Disinfesting Roots and Media of Potted Plants of Burrowing Nematodes. *Applied Engineering in Agriculture*. 17:533-538.

US Environmental Protection Agency. (December 1996). Methyl Bromide Alternative Case Study, Part of EPA 430-R-96-021, 10 Case Studies, Volume 2. Available on the web at: http://www.epa.gov/spdpublc/mbr/heatcom2.html

USDA Animal and Plant Health Inspection Service. 2001. Importing and Exporting Agricultural Products. USDA. Available on the web at: http://www.aphis.usda.gov/oa/getting.html

Wang, K.H. and B.S. Sipes. 1999. *Radopholus similis* in Anthurium Shoot Tissue. *HortScience*. 34:296-297.

Wang, K.K., Kuehnle, A.R., Sipes, B.S. 1998. *In vitro* tolerance and resistance to burrowing nematode, *Radopholus similis*, in *Anthurium* species. *Euphytica*. 103: 23-28.

Winfield, A.L. 1970. Factors affecting control by hot-water treatment of stem nematode *Ditylenchus dipsaci* (Kühn) Filipjev in narcissus bulbs. *Journal of Horticultural Science*. 45:447-456.

Yarwood, C.E. 1967. Adaptation of Plants and Plant Pathogens to Heat. *Molecular Mechanisms of Temperature Adaptation.* Prosser, C.L. (ed.), Am. Assoc. for the Advancement of Science: Washington D.C. pp. 75-89.

Roberts, P.A. and W.C. Matthews. 1995. Disinfection alternatives for control of *Ditylenchus dipsaci* in garlic seed cloves. *Journal of Nematology*. 27:448-456.

Tsang, M.M.C., A.H. Hara, and B.S. Sipes. 2001. Hot Water Drenching System Disinfesting Roots and Media of Potted Plants of Burrowing Nematodes. *Applied Engineering in Agriculture.* 17:533-538.

US Environmental Protection Agency. 1996. Methyl Bromide Alternative Case Study, Part of EPA 430-R-96-021, 10 Case Studies, Volume 2. Available on the web at: http://www.epa.gov/spdpublc/mbr/heatcom2.html

Wadley, F.M. 1949. Dosage-mortality correlation with number treated estimated from a parallel sample. *Annals of Applied Biology* 36:196-202.

CHAPTER 2

HOT WATER DRENCH TREATMENTS FOR THE CONTROL OF *RADOPHOLUS SIMILIS* IN *RHAPIS EXCELSA* AND *CARYOTA MITIS*

2.1. Abstract

Exporters of potted nursery stock face strict quarantine regulations against the burrowing nematode, *Radopholus similis*. Interceptions lead to significant economic loss and curtailment of trade. Currently, no treatments are approved to disinfest plants of *R. similis*. Hot water drench treatments were investigated for potential quarantine utility on commercially traded potted palms. *Rhapis excelsa* and *Caryota mitis* were inoculated with 5,000 mixed life stages of *R. similis* and allowed to establish for 14-weeks prior to drench treatments. The palms drenched in water at 50°C for period for 10 to 16 minutes. In *R. excelsa*, moderately good hosts to *R. similis*, a 16-minute hot water drench achieved 99.6% mortality of *R. similis*. In *C. mitis*, poor hosts to *R. similis*, all treatments longer than 10 minutes at 50°C eliminated *R. similis*. Probit regression estimate of the lethal temperature for 99% mortality (LT_{99}) for *R. excelsa* was 17.8 minutes. However, a Pearson χ^2 goodness-of-fit tests showed significant deviation from the estimates ($\chi^2 = 26.7$, df =2, $P<0.01$). The high efficacy of hot water drenches for the control of *R. similis* is approaching the Probit 9 standard of 99.9968% mortality required for USDA approval of hot water drenches as a quarantine treatment for *R. similis*.

2.2. Introduction

Exporters of potted nursery stock face strict quarantine regulations against Radopholus similis, the burrowing nematode. *Radopholus similis* is regulated by several states in the US, and by several countries in Europe, Asia, and Latin America (NPB, 2002). Interception of *R. similis* in a single plant will result in an entire shipment being returned to the grower or confiscated and destroyed at the point of entry. Currently, no procedure is approved to disinfest plants of *R. similis*. Consequently, worldwide trade of nursery stock is curtailed through inspection regimes, preshipment clearance certification programs, and rejections (Ishii *et al.*, 1956; Holtzmann *et al.*, 1984).

Research has been directed towards the therapeutic control of plant-parasitic nematodes with hot water immersions. Hot water immersion has effectively eliminated plant-parasitic nematodes in bulbs (Roberts and Matthews, 1995, Qiu *et al.*, 1993), propagative grapevine cuttings (Lear and Lider, 1959), and bare-root ornamentals (Birchfield and van Pelt, 1958). Hot water immersion is commercially infeasible for potted nursery stock because high efficacy can only be achieved by bareroot immersions. The process of bare rooting and repotting is time consuming and increases recovery period of treated plants (Tsang *et al.*, 2001). To address this problem, a continuous hot water drenching system was designed to rapidly and effectively deliver water at a target temperature to potted (Tsang *et al.*, 2001).

The thermal death point of *R. similis* was reported to be approximately 10 minutes at 50°C by Birchfield (1954). An identical treatment applied to control *Meloidogyne*

incognita revealed that efficacy varied among different plant species (Birchfield and Van Pelt, 1958). Since *R. similis* infects more than 365 species of plants (Holdeman, 1986), duration-temperature combinations of hot water treatments need to be determined on a case-by-case basis for each plant species. The objective of this study was to determine the optimal duration-temperature combination of hot water drenches for the control *of R. similis* in *Rhapis excelsa* and *Caryota mitis*.

2.3. Materials and Methods

Plant Materials and Growing Media

R. excelsa and *C. mitis* plants were obtained from commercial nurseries on the island of Hawaii. Plants were maintained in a shadehouse at the Waiakea Research Station, Hilo, HI. The growing media for both palm species was 1.3-cm crushed volcanic cinder and sphagnum peat moss (No. 4 Sunshine Mix, Sun Gro Horticulture, Canada) (60:40% by weight ratio of cinder to peat). *R. excelsa* and *C. mitis* palms were planted in 21-cm-diameter and 30-cm-diameter plastic pots, respectively.

Nematode Inoculation

R. similis was cultured in the laboratory on alfalfa callus (Ko, *et al.*, 1996). Nematodes were extracted using Baermann funnels and suspended in water (Barker, 1985). Five thousand mixed life stages of *R. similis* were delivered in 20-ml aliquots to each plant using a disposable pipette. All plants were inoculated 14-weeks before application of hot water treatments to allow nematode populations to establish. Plants were watered using overhead mist during this period.

Hot Water Drenching System

The constant temperature hot water drenching system consisted of a 340-L stainless steel reservoir, a water circulation and delivery system, and hot water heater (model PTH602, Omega Engineering, Stamford, CT) (Tsang *et al.*, 2001). Isothermal temperature was maintained in the reservoir by a thermostatically controlled shut-off valve (model SCR71-Z230, Omega Engineering, Stamford, CT) activated by a temperature controller (model CN77353, Omega Engineering, Stamford, CT) accurate to ±0.5°C. The unit drenches 4 pots simultaneously. The system was optimized to achieve a uniform and rapid rise to treatment temperature in the roots and media of plants (Tsang *et al.*, 2001).

Effects of Hot Water on Nematode Mortality

Forty 3-year-old *R. excelsa* were randomly assigned to exposure times of 0, 10, 12, 14, or16 minutes, 8 plants per treatment. Similarly, 32 4-year-old *C. mitis* were randomly assigned to exposure times of 0, 10, 13, or 16 minutes. The water temperature for all treatments was 50°C. Immediately after treatment, all plants were immediately cooled with a 25°C water drench for half of the treatment time.

Extraction Methods

Plants were assayed for surviving nematodes 7 days later. Stems, leaves, and petioles were discarded and roots were separated from the media and rinsed. Roots were chopped into 1-2 cm long pieces and fresh weight recorded. A 20-g subsample was taken from each plant and placed in a mist chamber for 3 days (Barker, 1985). The remaining

roots were placed in a 60 x 20 x 27.5-cm gusseted polyethylene bag and water added to cover half of the roots. Bags were maintained at 25°C in the dark for 7 days. After the incubation, water and roots were poured over a 20-µm mesh screen to collect nematodes. Samples were subjected to a density gradient and centrifuged to separate nematodes from fine soil and root particles (Barker, 1985). Bag samples yielded total number of nematodes per plant. From mist chamber samples, total number nematodes recovered per plant was calculated using the formula: $(N_t \times F_{wt})/20g$, where N_t is the number of *R. simlis* recovered and F_{wt} is the total fresh weight of roots.

Data Analysis

All *R. similis* recovered, regardless of their vitality, were presumed to be survivors of the hot water treatment because nematodes succumbing to heat treatments were rinsed away before extraction. *R. similis* and microbiverous nematodes were counted using an inverted light microscope (Leica DM/IRB®, Leica Microsystems, Inc.). Statistical analysis consisted of a nested analysis of variance for extraction method within duration of exposure. Arcsine transformation of percentage mortality was used to adjust non-normal distribution of data. Single degree of freedom contrasts were used to identify differences among treatments. Probit analysis was performed to estimate a time exposure lethal to 99% of treated *R. similis* (LT_{99}), in *R. excelsa*.

2.4. Results

Comparison of Extraction Methods

Bag extractions yielded *R. similis* in more treatments and in higher numbers than did mist extraction (Table 1). In *R. excelsa*, mist extraction failed to detect *R. similis* in exposure treatments of 12-minutes or longer, while bag extraction recovered *R. similis* in all treatments. In *C. mitis*, mist extraction recovered *R. similis* only in the control treatment whereas bag extraction detected *R. similis* in the control and 10-minute treatments. Although the level of difference between extraction method was not significant for *C. mitis* ($P = 0.09$) or for *R. excelsa* ($P = 0.46$), bag extraction consistently detected *R. similis* in a higher frequency of replications and in higher numbers at all levels of treatment. All subsequent statistical analysis was performed on data obtained from bag extractions.

Microbiverous and non-target plant-parasitic nematodes recovered from bag extractions were also detected at an equal or higher frequency than in mist extractions, in both species and in all treatments except the 14-minute treatment on *R. excelsa* (Table 1). Other nematode species identified in samples were *Aphelenchoides spp.*, *Meloidogyne spp.*, *Xiphinema spp.*, *Rotylenchulus reniformis*, *Criconemella spp.*, and several species in the Rhabtidae. No direct relationship between longer treatments and mortality was observed with this group (Table 1).

Table 2.1. Presence of *Radopholus similis*, microbiverous, and non-target plant-parasitic nematodes in palms using bag and mist extraction methods.

		R. similis				Microbiverous/ non-target plant-parasitic nematodes			
		Bag		Mist		Bag		Mist	
Palm Species	Duration at 50 C	%[a]	No[b]	%	No.	%	No.	%	No.
Rhapis excelsa	0	100	110	25	14	100	300	100	179
	10	25	26	25	11	88	74	63	32
	12	71	11	0	0	75	146	63	58
	14	50	4	0	0	75	6	50	58
	16	13	1	0	0	38	76	50	41
Caryota mitis	0	14	3	14	6	100	2578	100	1923
	10	25	2	0	0	100	756	63	66
	13	0	0	0	0	100	262	88	243
	16	0	0	0	0	88	338	75	146

[a] Percent of replications with survivors (n = 8)
[b] Mean of survivors from 8 plants

Efficacy of Hot Water Drenches

A positive relationship between exposure to hot water and mortality of *R. similis* was documented in both palms (Fig. 1). Starting populations were estimated, following a maximum likelihood solution, by using the average of survivors from the control group (Wadley, 1949). Percent mortality was calculated as 1 minus the total number of *R. similis* divided by the mean of *R. similis* recovered from control treatment. The mean population in the control treatment was 110 per plant for *R. excelsa* and 3 per plant from *C. mitis* (Fig. 1).

In *R. excelsa*, the 16-minute drench treatment reduced *R. similis* populations by 99.6% when compared to the mean of the 0-minute control treatment. Only one replicate in the 16-minute exposure treatment contained *R. similis*. Single-degree-of-freedom contrasts showed differences between control and all other treatments ($P<0.01$) and between control and the 16-minute treatment ($P<0.01$) but there were no differences among the 10-, 12-, 14-, or 16- minute treatments.

In *C. mitis*, all *R. similis* were eradicated from plants treated longer than 10-minutes (Table 1). The poor host status of *C. mitis* palms to *R. similis* was confirmed (Goo and Sipes, 1999). Single degree of freedom contrasts among treatments from bag extractions data detected no difference among treatments ($P = 0.48$), although complete control was observed in all replicates from 13- and 16-minute treatments (Fig. 1).

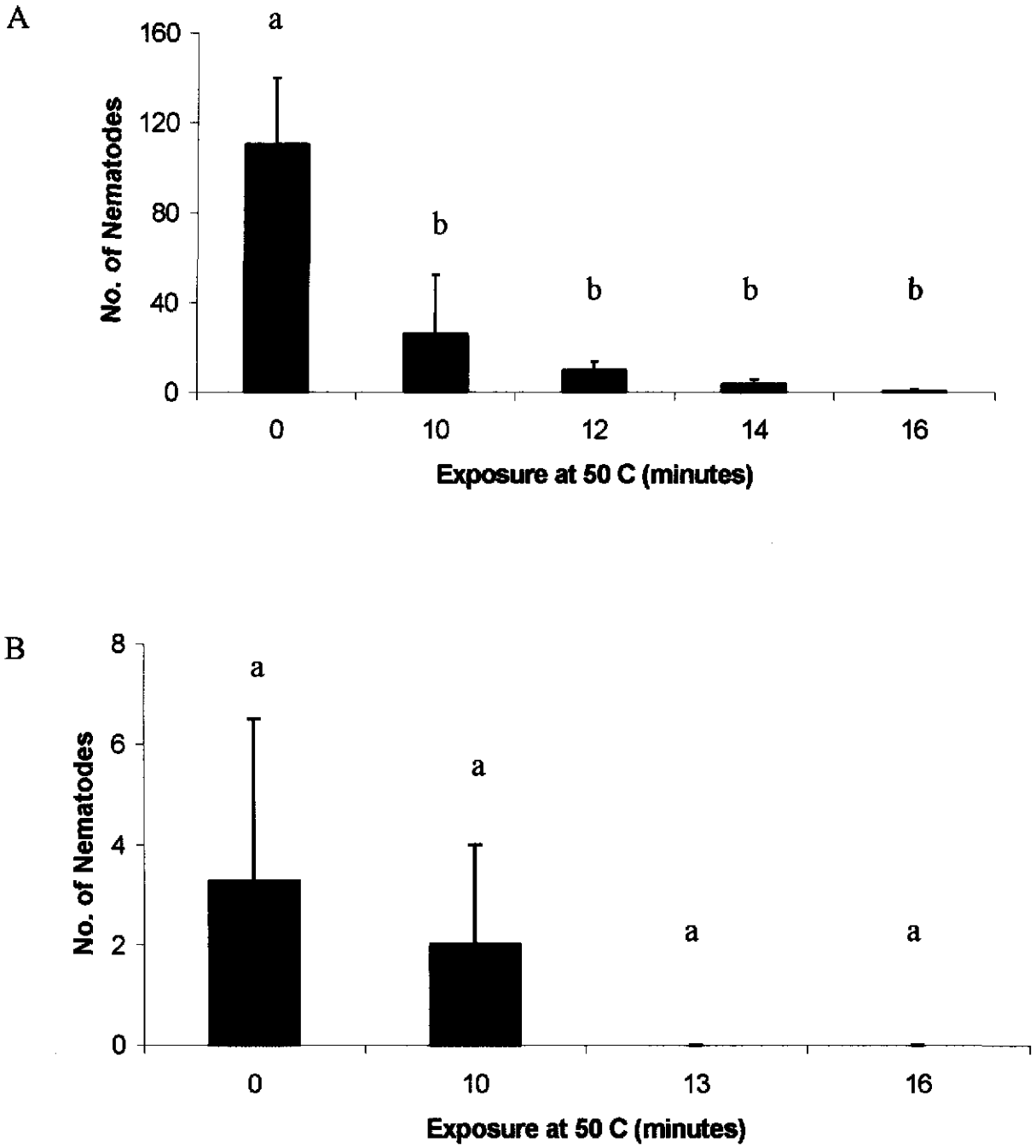

Figure 2.1. Mean number of *Radopholus similis* detected by bag extraction in (A) *Rhapis excelsa* and (B) *Caryota mitis* (n=8). Error bars represent standard error. Bars with same letters are not significantly different ($P > 0.05$).

Since *R. similis* was not eradicated in *R. excelsa*, probit analysis was used to estimate a probit regression line with 95% fiducial limits (Finney, 1971) (Fig. 2). Lethal time (LT) for any level of mortality can be estimated from this probit regression line. The LT_{99} probit estimate for *R. similis* mortality was 17.8 minutes with a 95% lower and upper confidence interval of 16.5 and 19.8 minutes, respectively. However a Pearson χ^2 goodness of fit test conducted on deviation of observed data from probit regression estimates was significant ($P<0.01$). The high χ^2 value usually suggests an inappropriate model, however since treatments were designed to achieve complete control, probit regression estimates were compromised by lack of data at lower mortality rates.

2.5. Discussion

Hot-water immersion and other thermotherapy treatments such as hot air and hot water vapor are USDA-APHIS approved for quarantine treatment of economically important tropical fruits for various Tephritidae fruit fly species (U.S. EPA, 1996). Results clearly delineate the temperature/time combinations that will be required for employing hot water drenches in palms for the eradication of *R. similis* from plant tissue. Quarantine protocols accepted by the USDA are based on the probit 9 (99.9968% mortality) security level, which equates to 32 survivors out of one million treated individuals. The low observed infestation levels of *R. similis*, in *R. excelsa* and *C. mitis* would require inordinate number of replications. Approximately 9,000 *R. excelsa* plants would be required to treat one million *R. similis* individuals, thus detection at the probit 9-security level (± 0.0032%) would require an unrealistic experimental design. Attempts

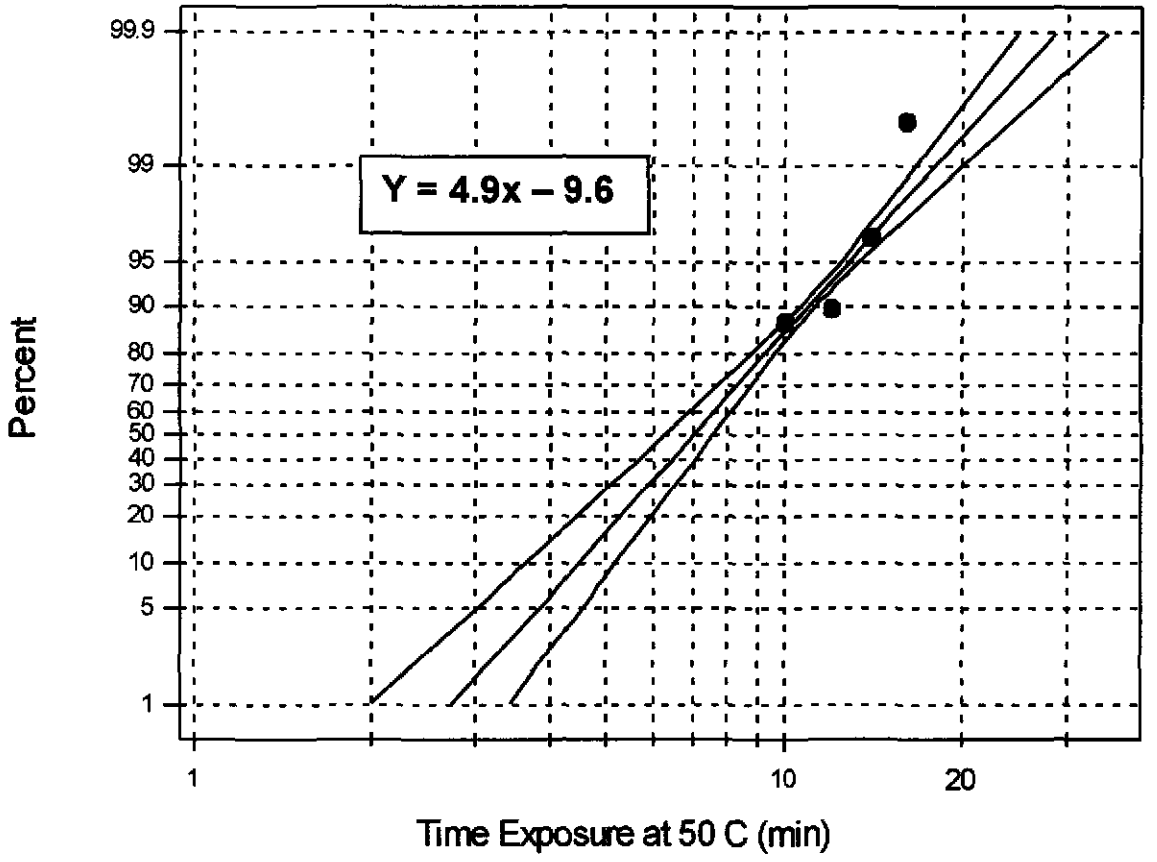

Fig. 2.2. Probit regression estimate for mortality of *Radopholus similis* at 50°C with 95% lower and upper confidence intervals in *Rhapis excelsa*. Observed values are plotted as (•). Linearity of exponential data is achieved by transforming time (x-axis) to ln(time) and percent mortality (y-axis) to ln(%/1-% mortality). Non transformed values are plotted on log scale axes for clarity. Deviation from observed data using a Pearson χ^2 goodness-of-fit is significant ($P < 0.01$). Distribution of probit estimates was assumed to be loglogistic.

to modify Probit-9 security level requirements to account for low infestation levels of some pathogen-commodity complexes are ongoing at quarantine administration level (Follett and McQuate, 2001). Until such modification, a more pragmatic approach is to develop treatments that achieve complete control consistently. The mortality of *R. similis* from the longer exposure treatments achieves complete control in *C. mitis* and a very high level in *R. excelsa*. Although statistical modeling can be employed to predict a treatment level expected to achieve complete control, design of treatments should be evenly spaced to give robust data for a wide range of mortality (Finney, 1958).

By establishing hot water drenches as a procedure that can eradicate *R. similis*, we propose their acceptance as acceptable quarantine treatments. Adoption by industry will be subject to cost-benefit analysis, which is unlikely to occur without acceptance of the treatment by quarantine authorities. The economic impact of developing a therapeutic treatment for *R. similis* in infested potted ornamentals will be increased efficiency in the international and interstate trade of these commodities. Furthermore, application of these treatments on propagative material during production will further reduce spread, lower infestation rates, and improve plant growth.

2.6. Literature Cited

Barker, K.R. 1985. Sampling Nematode Communities. *An Advanced Treatise on Meloidogyne Vol II Methodology*. Barker, K.R., C.C. Carter, J.N. Sasser (eds). North Carolina State University Graphics: Raleigh, N.C. pp.25-28.

Birchfield, W. 1954. The hot-water treatment of nematode-infested nursery stock. *Proceedings of the Florida State Horticulture Society*. 67:94-96.

Birchfield W. and H.M. van Pelt. 1958. Thermotherapy for nematodes of ornamental plants. *Plant Disease Reporter*. 42:451-455.

Finney, D.J. 1971. *Probit Analysis*. 3rd Edition. Cambridge University Press: Cambridge, UK. pp. 50-80, 202-211.

Follett, P. A., and G. T. McQuate. 2001. Accelerated quarantine treatment development for insects on poor hosts. *Journal of Economic Entomology*.. 94(5): (Forum section)

Goo, M.Y.C. and B.S. Sipes. 1999. Chromosome number and reproductive isolates of *Radopholus similis* from Hawaii. *International Journal of Nematology*. 9:43-46.

Holdeman, Q.L. 1986. The burrowing nematode, *Radopholus similis*, sensu lato. California Dept. of Food and Agriculture, Division of Plant Industry, Sacramento. p. 13.

Holtzmann, O.V., A.P. Martinez, W.J. Apt. 1984. Burrowing Nematodes: A menace to Hawaii nurseries. Honolulu: Hawaii Inst. of Trop Ag. and Human Res. Univ. of Hawaii Info. Text Series. 20.

Ishii, M., H. Kamemoto, T.K. Maeda, R.K.T. Au. 1956. Investigations on the control and distribution of the Burrowing Nematode. Final Report to Hawaii Economic Planning and Coordination Authority. Grant no. 5. State of Hawaii.

Ko, M.P. D.P. Schmitt, and B.S. Sipes. 1996. Axenizing and culturing endomigratory plant-parasitic nematodes using Pluronic F127, including its effects on population dynamics of *Pratylenchus penetrans. Journal of Nematology* 28:115-123.

Lear, B. and L.A. Lider. 1959. Eradication of root-knot nematodes from grapevine rootings by hot water. *Plant Disease Reporter* 43:314-317.

National Plant Board. *State and Federal Quarantine Summaries.* Compiled by USDA/APHIS. 1 October 2002. <http://www.aphis.usda.gov/npb/F&SQS/sqs.html>

Qiu, J., B.B. Westerdahl, D. Giraud, and C.A. Anderson. 1993. Evaluation of hot water treatments for management of *Diteylenchus dipsaci* and fungi in daffodil bulbs. *Journal of Nematology* 25:686-694.

Roberts, P.A. and W.C. Matthews. 1995. Disinfection alternatives for control of *Ditylenchus dipsaci* in garlic seed cloves. *Journal of Nematology*. 27(4):448-456.

Tsang, M.M.C., A.H. Hara, and B.S. Sipes. 2001. Hot Water Drenching System Disinfesting Roots and Media of Potted Plants of Burrowing Nematodes. *Applied Engineering in Agriculture.* 17:533-538.

US Environmental Protection Agency. 1996. Methyl Bromide Alternative Case Study, Part of EPA 430-R-96-021, 10 Case Studies, Volume 2. <http://www.epa.gov/spdpublc/mbr/heatcom2.html>

Wadley, F.M. 1949. Dosage-mortality correlation with number treated estimated from a parallel sample. *Annals of Applied Biology.* 36:196-202.

CHAPTER 3

LOCALIZATION *RADOPHOLUS SIMILIS* IN *ANTHURIUM* AFTER HOT WATER DRENCH TREATMENT

3.1. Abstract

Live *Radopholus similis* is present in *Anthurium* tissue 4-weeks after drenches of 10-15 minutes in 49°C water. This experiment was designed to locate the survivors by partitioning treated *Anthurium* tissue. One week after treatment at 49°C 12-minute *R. similis* was recovered only from stem tissue above the soil level one plant. Populations were reduced by 99.997% when compared to the 25°C control treatment. Four weeks after treatment, nematodes were detected in stems above the soil line, stems below the soil line, and distal 4-cm of roots. When the above ground stems were removed immediately after the hot water treatment, *R. similis* survivors were not detected at 1- or 4-weeks after treatment. Since nematodes were initially recovered only from untreated tissue, it is most probable survivorship is linked to presence of nematodes in untreated tissue and subsequent migration, rather than a high heat tolerance of *R. similis*.

3.2. Introduction

Potted Anthurium plants drenched with 49 °C water for 10 or more minutes can be disinfested of *Radopholus similis* (Sipes and Hara, unpublished). However 2-months after treatment, some treated *Anthurium* were infected with low numbers of nematodes in roots and stems (Sipes and Hara, unpublished). The USDA probit 9 security level maximum of 32 survivors in a million treated individuals (99.9968% mortality) (US EPA, 1996) required for approval of quarantine treatments was not being met. The

development of a therapeutic quarantine treatment for ornamentals to eradicate *R. similis* can decrease economic losses incurred by industry when interceptions occur.

Surviving nematodes in following hot water drenching of *Anthurium* is indicative of insufficient control, escapes, or reinfestation. Contamination of disinfested plants from the surrounding environment during the observation period could have occurred. Long distance nematode dissemination is often due to passive dispersal by wind, irrigation, or human activity (Prot, 1986). However, hot water drenched plants were maintained in greenhouses on sterilized benches with drip irrigation, which reduced the potential for long distance movement from pot to pot, or from bench to bench. Inadequate control or escapes are the most probable reason that live *R. similis* are recovered after a hot water treatment.

Stem and petioles 6 cm or greater above growth medium of potted *Anthurium andraeanum* was reported to contain *R. similis* (Wang and Sipes, 1999). Hot water drench treatments do not expose more than the lower 1-2 cm of stems directly to hot water. Much of the stems remain unexposed to target treatment temperatures thus presenting a possible mechanism for nematodes to reinfest stems and roots through migration.

Vermiform *R. similis* or eggs or also may have withstood the treatment temperatures in insulated pockets in media or roots. The location of any surviving nematodes in treated plants must be established to determine whether the presence of survivors is due to migrations within *Anthurium* tissue or there is a systematic failure of hot water drenches to eradicate nematodes in roots and media. If surviving nematodes

are due to migrations from untreated stem tissue, modifications of hot water drench treatment will be necessary to eradicate nematodes in the stem. The objective of these experiments was to determine the location of *R. similis* surviving a hot water drench in *Anthurium* tissue one and four weeks after treatment.

3.3..Materials and Methods

Two experiments were conducted in a greenhouse to determine location of *R. similis* in roots and stems of *Anthurium.* In the first experiment 40 plants each of *Anthurium* cultivars, 'Tropic Fire,' 'Misty Pink,' 'Lady White,' and 'Waimea,' were grown in plastic 15-cm-diameter pots filled with a 3-cm crushed volcanic cinder and sphagnum peat moss (No. 4 Sunshine Mix, Sun Gro Horticulture, Canada) media in a 3:2 (cinder:peat) ratio by weight. *R. similis* inoculum were cultured on alfalfa callus tissue and extracted 24 hours before inoculation (Ko *et al,* 1996). All plants were inoculated with 2,000 mixed life stages of *R. similis* 9-weeks before treatment. The plants were lightly watered during the first 2 weeks after inoculation to avoid leaching nematodes. Experimental design was a split plot factorial with the main plot being temperatures of 49°C and 25 °C, each for 12 minutes. The subplots consisted of two sample times: 1- and 4-weeks after treatment. Each treatment combination was replicated 10 times and repeated for each cultivar. All plants were drenched using a recirculating hot water drenching system (Tsang, *et al.,* 2001). Immediately after drenching, the plants were cooled with ambient water for 6 minutes. Plants were maintained in a greenhouse during

the observation period and splashguards were placed between plots to avoid cross contamination.

One week after treatment, a set of plants was assayed for surviving nematodes. The potting media were separated from the roots. The plants were divided into sections of the distal 4-cm of the root mass, roots in the medial 4-cm-of root mass, stem below soil surface, and stem portions above the soil surface. All plant partitions were chopped into 3 to 5-cm pieces, fresh weight recorded, and 20-g subsamples placed in a mist chamber for 3 days to extract nematodes (Barker, 1985). Nematodes were counted using an inverted light microscope (Leica DM/IRB®, Leica Microsystems, Inc.). Nematodes were considered viable by presence of motility when prodded. Four weeks after treatment, the remaining plants were assayed for nematodes using the same method.

Since large differences were expected between 49°C and 25°C drench water treatments and also between one- and four- week populations after treatment, a one-way analysis of variance, rather than a full factorial analysis, was conducted to determine whether there were differences in cultivar susceptibility to *R. similis*. Analysis of variance was conducted on whole plant data (partitions combined) from the control 25° drench treatments 4-weeks after treatment. These data represented the maximum incubation period for nematodes in this experiment and was used to ascertain relative susceptibility of the four cultivars. Population of *R. similis* was measured by a reproductive factor (R_f) calculated by $\log(R_f + 1)$, where R_f was final nematode population divided by inoculum level.

In a second experiment, 60 *Anthurium* cv. 'Waimea', planted in 15-cm-diameter-plastic pots, were inoculated with 2,000 mixed life stages of *R. similis,* collected from alfalfa callus cultures (Ko *et al,* 1996) 14-weeks before hot water treatment. Treatments were arranged in a 2 x 2 factorial design with 2 levels of drench, 49°C and 25°C for 12 minutes and 2 levels of observation periods, 1 and 4 weeks after drench. Immediately after drenching all plant tissue that did not receive direct exposure to hot water drench, ie. stem tissue above the level of the pot rim, was removed and assayed for nematodes. The 4-week nematode assay was conducted as described with all plants being partitioned into four sections, except that stem above the soil line partition now only consisted of stem tissue that received direct exposure to hot water drenches.

3.4. Results

Analysis of variance to determine relative cultivar susceptibility resulted in no differences ($P = 0.95$) (Fig. 1). Furthermore cultivars that contained the highest level of nematode populations (Rf) from the 25° water treatment 4-weeks after treatment did not correspond with the cultivars that contained the survivors (Table 1).

Survivors were detected only in the untreated stem portion above the soil line from one replicate of 'Waimea' at one week after treatment (Table 1). The 49°C drench achieved a mortality of 99.997% of the estimated treated population (Wadley, 1949). One hundred percent mortality was observed in all plant partitions that received direct exposure to the hot water drench (Table 2).

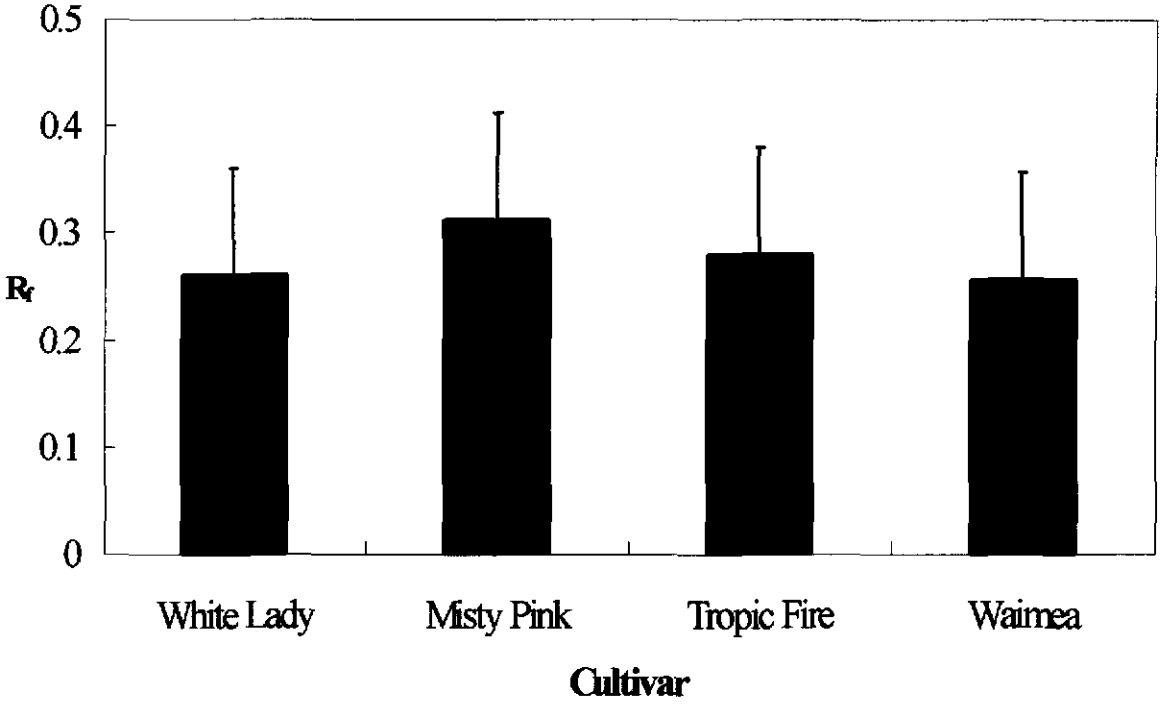

Fig. 3.1. Mean of $\log(R_f+1)$, where R_f is *Radopholus similis* final population/initial population inoculum from four *Anthurium* cultivars. Final population was the sum of all plant partitions after a control, 25°C water drench treatment, and were sampled 18 weeks after inoculation. Differences in cultivar R_f were not signifcant ($P > 0.05$).

Table 3.1. Location and cultivar from which surviving *Radopholus similis* were recovered in *Anthurium* plants drenched with 49°C for 12-minutes and assayed 1- and 4-weeks after treatment.

Plant partition[a]	Cultivar	Weeks after treatment	Cultivar Ranking[b]
Above Stem	Waimea	1	3
Above Stem	Tropic Fire	4	2
Below Stem	Misty Pink	4	4
Distal Roots	White Lady	4	4

[a] Distal Roots = Distal 4-cm-diameter of roots, Above Stem = 5-cm of stem above soil surface, Below stem = all stem parts below soil surface.

[b] Ranking: 1=highest average number of nematodes, 4 = lowest average number of nematodes in control group, within plant partition, among four cultivars.

At 4-weeks after treatment, nematodes were detected in all plant partitions except in the medial roots. Low numbers of survivors were collected from cultivars, 'Tropic Fire,' 'Misty Pink,' and 'White Lady', 4-week after treatment (Table 1).. At 4-weeks after treatment overall efficacy of hot water drench was 99.99964% with a total of 30 surviving nematodes compared to of 83,574 recovered from the 25°C water treatment (Table 2).

Table 3.2. Efficacy of a hot water drench at 49°C for 12 minutes in different plant partitions of *Anthurium* 1- and 4-weeks after treatment.

Weeks after treatment	Plant partition[a]	Efficacy within plant partition(%)[b]	Efficacy with partitions combined(%)[c]
1	Above Stem	98.24	99.99738
	Below Stem	100.00	
	Medial Roots	100.00	
	Distal Roots	100.00	
4	Above Stem	99.52	99.99964
	Below Stem	99.85	
	Medial Roots	100.00	
	Distal Roots	99.95	

[a] Above stem = 5-cm of stem above soil surface, Below stem = all stem parts below soil surface, Medial Roots = medial 4-cm-diameter of roots, Distal Roots = distal 4-cm-diameter of roots.

[b] Efficacy calculated by $1 - (N_s/N_c) \times 100\%$, where N_s = mean number of nematode survivors and N_c = mean number of nematodes in the 25° treatment group (data from 4 cultivars combined to calculate means, n =40).

[c] Efficacy calculated by $1-(Ns/Nc) \times 100\%$, where N_s = mean number of nematode survivors from all plant partitions (n=40) and N_c = mean number of nematodes in the 25° treatment group (all plant partitions and cultivars combined, n=40).

In the second experiment removing the untreated stems immediately after drenching increased the efficacy of hot water treatments. Complete control was achieved and no survivors were detected at 1- or 4- weeks after treatment (Table 3).

Table 3.3. Number of *Radopholus similis* in plant partitions of *Anthurium* cultivars 'Waimea,' 1- and 4-weeks after a 49°C 12-minute hot water drench.

Plant partition[b]	Nematodes[a]				
	Immediately after treatment	1-week		4-weeks	
		25°C/12min	49°C/12min	25°C/12min	49°C/12min
Stem	0	--	--	--	--
Distal Roots	--	40 ± 16	0 ± 0	52 ± 16	0 ± 0
Medial Roots	--	43 ± 16	0 ± 0	60 ± 23	0 ± 0
Below Stem	--	12 ± 9	0 ± 0	80 ± 34	0 ± 0
Above Stem	--	0 ± 0	0 ± 0	7 ± 7	0 ± 0

[a] Numbers reported are means of 15 replications with standard error of the mean.

[b] Stem is all stem portions (excluding leaves, flowers, petioles) 5-cm above soil line. Above Stem = 5-cm of stem above soil surface, Below Stem = all stem parts below soil surface, Medial Roots = medial 4-cm-diameter of roots, Distal Roots = outer 4-cm-diameter of roots.

3.5 Discussion

A primary concern from past experiments was the appearance of few survivors 2-months after treatment in a few plants. Whether this indicated a systematic failure of the hot water treatments or reinfestation of otherwise disinfested roots was investigated by partitioning plants before assay. One week after treatment with hot water nematode survivors were located only in the stem section above the soil line, a portion of the plant that received only minimal exposure to target treatment temperatures. Four weeks later, nematodes were detected in hot water treated root and stem tissue. The possibility of live *R. similis* in the untreated stems migrating and recolonizing previously treated and disinfested root tissue is positively established.

Migration of plant-parasitic nematodes may be random or directed by signals emitted from food sources or sex partners (Samoiloff *et al.*, 1994). Nematodes are thermotactic and applying hot water to roots stimulates migration. *Meloidogyne incognita* (Diez and Dusenbery, 1989) and *Caenorhabditis elegans* (Mori and Oshima, 1995), can be sensitive to temperature and migrate from extreme to moderate temperatures.

When stems were removed at the pot rim level of treated Anthurium plants, preventing possible recolonization of disinfested roots, hot water drenches achieved 100% control, indicated by the absence of nematodes in assays 1- and 4-weeks after treatment. *R. similis* were not recovered from the untreated stems removed immediately after treatment suggesting a population had not established in this tissue and recolonization would not have been possible. However, in the 25°C treated plants 4-

weeks after treatment, specimens of *R. similis* were recovered from the short stubs of above ground stems that remained. Many factors could have influenced the presence of nematodes in the stem including but not limited to, time allowed for establishment, random migration, or population pressure in the roots. Establishment period was increased from 9 weeks in the first experiment to 14 weeks in the second experiment so time for establishment could be evaluated as a factor as well as time of year for each experiment. Failure to recover *R. similis* in the stems, from the second experiment, could be due, in part, to less nematode reproduction in the second experiment than in the first, so there was much less population pressure forcing migration.

No evidence was found that correlated escapes or survivors with differences in relative susceptibility of the cultivars used in this experiment. Relative reproductive rates of *R. similis* were similar among cultivars. The similarity in susceptibility was expected since breeding objectives for commercial cultivars are mainly for horticultural attributes such as flower size or color, so detectable resistance to *R. similis* would be incidental (Kamemoto and Kuehnle, 1996). The fact that most *Anthurium* are susceptible to *R. similis*, indicate that resistance is infrequent among current cultivars (Wang, *et al.*, 1998).

Other mechanisms for survival must be considered. Thermal tolerance of vermiform *R. similis* or eggs to a 49°C hot water drench treatment or escapes facilitated by insulating properties of root tissue and media were not addressed by this study. Tolerance of plant-parasitic nematodes to high heat temperatures are documented in *Ditylenchus dipsaci* and *Anguina tritici* through a process of anhydrobiosis (cryptobiosis) (Norton, 1978). However, other than anhydrobiotic nematodes, there is no evidence that

fully hydrated juveniles or adults can be induced to dormancy by elevated temperatures (Womerseley, *et al.*, 1998). Quiescence of eggs resulting from elevated temperatures exists in *Meloidogyne javanica* and *M. naasi*, however the eggs of these species are laid in an egg sac which is partially enclosed in a root gall induced by feeding *Meloidogyne* spp. females (Antoniou, 1989). *R. similis* does not have such specialized egg protective material.

Identification of *R. similis* in the stem after a hot water drench will require a modification of hot water treatment to raise stem tissue to target temperatures. Eradication of *R. similis* from all plant tissue will likely be necessary for approval or treatment may have to be limited to cultivars which prevent nematode movement into stem tissue.

3.6 Literature Cited

Antoniou, M. 1989. Arrested development in plant-parasitic nematodes, *Helminthological Abstracts B* 58: 1-19.

Barker, K.R. 1985. Sampling nematode communities. *An Advanced Treatise on Meloidogyne Vol II Methodology.* Barker, K.R., Carter, C.C., Sasser J.N. (eds). North Carolina State University Graphics: Raleigh, N.C.

Diez, J.A. and D.B. Dusenbery. 1989. Preferred temperature of *Meloidogyne incognita. Journal of Nematology* 21:99-104.

Fielding, M.J. 1951. Observations on the length of dormancy in certain plant infecting nematodes. *Proceedings of the Helminthological Society* 18:110-112.

Kamemoto, H. and A.R. Kuehnle, 1996. Breeding *Anthurium* in Hawaii. University of Hawaii Press, Honolulu. 132 pp.

Ko, M.P. D.P. Schmitt, and B.S. Sipes. 1996. Axenizing and culturing endomigratory plant-parasitic nematodes using Pluronic F127, including its effects on population dynamics of *Pratylenchus penetrans. J. of Nematology.* 28(1):115-123.

Mori, I. And Y. Ohsima. 1995. Neural regulation of thermotaxis in *Caenorhabditis elegans. Nature* 376:344-348.

Norton, D.C. 1978. *Ecology of Plant-Parasitic Nematodes.* Wiley and sons, New York, 268 pp.

Prot, J.C. 1986. Migration of plant-parasitic nematodes towards plant roots. *Revue de Nematologie* 3:305-318.

Samoiloff, M.R., S. Balakanich, and M. Petrovich. 1974. Evidence for the two-state model of nematode behaviour. *Nature* 247:73-74.

Tsang, M.M.C., A.H. Hara, and B.S., Sipes. 2001. Hot water drenching system disinfesting roots and media of potted plants of burrowing nematodes. *Applied Engineering in Agriculture* 17:533-538.

US Environmental Protection Agency. (December 1996). Methyl bromide alternative case study, Part of EPA 430-R-96-021, 10 Case Studies, Volume 2.

Wadley, F.M. 1949. Dosage-mortality correlation with number treated estimated from a parallel sample. *Annals of Applied Biology* 36:196-202.

Wang, K.K., A.R. Kuehnle, and B.S. Sipes. 1998. *In vitro* tolerance and resistance to burrowing nematode, *Radopholus similis*, in *Anthurium* species. *Euphytica* 103:23-28.

Wang, K.H. and B.S. Sipes. 1999. *Radopholus similis* in anthurium shoot tissue. *HortScience* 34:296-297.

Womerseley, C.Z., D.A. Wharton, and L.Y. Higa. 1998. Survival Biology. *The Physiology and Biochmeistry of Free-living and Plant-Parasitic Nematodes.* Eds. R.N. Perry and D.J. Wright. New York: CABI Publishing. 288-291.

CHAPTER 4

THE EFFECT OF HEAT CONDITIONING OF *ANTHURIUM* ON EFFICACY OF HOT WATER DRENCH TREATMENTS FOR THE CONTROL OF *RADOPHOLUS SIMILIS*

4.1. Abstract

Heat conditioning treatments applied to *Anthurium* to improve thermotolerance to hot water drenches applied for the control *Radopholus similis* may also allow development of thermotolerance in nematodes. Tests were conducted to determine whether applying conditioning treatments decreases efficacy of eradication treatment. *R. similis* were conditioned at 35°C, 40°C, and 45°C for 0, 15, 30, 60, 120, and 180 minutes *in vitro* then subjected to 47°C for 5 minutes. No nematodes survived the challenge heat treatment. The roots of inoculated *Anthurium* plants conditioned at 40°C for 15 minutes and unconditioned plants were subsequently treated at 45°C for 0 to 8 minutes. Probit analysis was used to compare LT_{50} and parameters of probit regression estimates for mortality. A χ^2 test for equal slopes was not significant ($P = 0.85$) and LT_{50} values were 55 and 56 seconds for conditioned and unconditioned *R. similis* respectively. In *Anthurium* plants conditioned at 40°C for 15 minutes using a hot water drench system and challenged with a lethal treatment of 49°C for 15 minutes, few nematodes survivors were detected in an assay 1-week after treatment. The presence of *R. similis* survivors in conditioned *Anthurium* does not presume that thermotolerance was induced by nonlethal heat. Without corroborating evidence such as protein analysis for heat shock proteins, other mechanisms such as thermotaxis during the nonlethal conditioning should be

considered. Heat conditioning treatments of *Anthurium* should not be applied since efficacy of subsequent hot water drenches is seriously compromised.

4.2. Introduction

Hot water drench treatments of ornamentals infested with *Radopholus similis* are highly effective and currently the only therapeutic option for plants destined for export markets. In order to decrease phytotoxic effects of heat and increase heat tolerance of potted *Anthurium*, conditioning treatments are being tested for heat susceptible cultivars. Plants and fruits acquire transient thermotolerance when briefly exposed to non-lethal high temperature prior to challenge heat temperature (Yarwood, 1961, Chan and Linse, 1989, Burke *et al.*, 1984, Woolf and Lay-Yee, 1997). These conditioning treatments improve postharvest quality and also increase efficacy of subsequent heat treatments. However, the synthesis and accumulation of heat shock proteins (HSP), the mechanism that confers thermotolerance in plants, has also been identified in *Caeonorhabditis elegans* (Snutch and Baillie, 1983) and *Heterohabditis bacteriophora* (Selvan *et al.*, 1996). The objective of this study is to determine whether conditioning treatments increased *R. similis* survival of a lethal hot water drench treatment of 49°C for 12 minutes.

4.3. Materials and Methods

In vitro Conditioning

An *in vitro* test of aqueous suspensions of *R. similis* conditioned with nonlethal heat temperatures then challenged with a subsequent lethal heat treatment was conducted.

All nematodes were cultured on alfalfa callus tissue (Ko *et al,* 1996). Nematodes were extracted 24 hours prior to the experiment using Baermann funnels, counted, and suspended in water at a density of 100,000 mixed life stages/l (Barker, 1985). One-ml aliquots containing approximately 100 nematodes were pipetted into 1.5 ml Epindorf tubes (Quality Scientific Plastics®) placed in a dry bath incubator (Fisher Scientific®) and heated to treatment temperature. Duration of treatment was measured only when aliquots reached treatment temperature.

Prior to testing conditioning treatments, a lethal death point temperature for *R. similis, in vitro* was determined by subjecting nematodes to 43, 45, 47, and 49°C for 0, 1, 2, 4, 6, 8, 10, 12, and 15 minutes. Each temperature/duration combination was replicated 5 times and the entire experiment repeated once. After treatment, aliquots were store in a 25°C agitated ambient water bath until counted using an inverted light microscope (Leica DM/IRB®, Leica Microsystems, Inc.) Mortality was determined by absence of motility when prodded. Data were arcsine transformed and tested for homogeneity of variance. Data were combined from the two repetitions where appropriate. Time to kill 99.9% ($LT_{99.9}$) of the nematodes was calculated using probit analysis.

Conditioning treatments of 35, 40, and 45°C for 0, 15, 30, 60, 120, and 180 minutes were tested for their ability to confer thermotolerance against an *in vitro* lethal treatment of 47°C for 5 minutes. Nematodes were raised on alfalfa callous tissue (Ko *et al,* 1996). Twenty-four hours before the experiment, nematodes were extracted using Baermann funnels, counted and suspended in water at a density of 100,000 mixed life stages/L (Barker, 1985). One-mL aliquots containing approximately 100 nematodes were

pipetted into 1.5ml Epindorf tubes (Quality Scientific Plastics®). Aliquots were placed in a dry bath incubator (Fisher Scientific®) and heated to treatment temperature. Duration of treatment was measured only when aliquots reached treatment temperature. Each conditioning treatment was replicated 5 times and the experiment was repeated once. After conditioning, nematode suspensions were place in an agitated 25°C bath for a lag period of 3 hours. After the lag period, aliquots were returned to the dry bath incubator for a lethal challenge heat treatment of 47°C for 5 minutes. After challenge heat treatment, aliquots were returned to the 25°C ambient water bath until counted using an inverted light microscope (Leica DM/IRB®, Leica Microsystems, Inc.). All data were arcsine transformed to test homogeneity of variance and data from repetitions combined when appropriate.

Anthurium Conditioning

Conditioning effects on nematode survival after a hot water drench were also tested in *Anthurium*. Twenty *Anthurium andraeanum* cv. 'Mickey Mouse' were planted in 15-cm plastic pots filled with a mix of 3-cm crushed volcanic cinder and sphagnum peat moss (No. 4 Sunshine Mix, Sun Gro Horticulture, Canada) media 3:2 (cinder:peat). All plants were inoculated with 10,000 mixed life stages of *R. similis*. After an establishment period of 17-weeks, plants were conditioned with a 40°C water drench for 15 minutes. Drenches were applied with a continuously recirculating hot water unit designed to maintain isothermal temperatures in the plant roots and media (Tsang *et al.*, 2001). A 3-hour lag period at 27°C followed the conditioning treatment. Ten plants

were challenged with a 49°C drench for 12 minutes. Ten plants received a 24°C water drench for 12 minutes. The 49°C treated plants were cooled for 6 minutes with an ambient water drench. All plants were assayed for nematodes 1-week after treatment. The potting media were separated from the roots. Roots were chopped into 3- to 5-cm pieces, weighed, and placed in a mist chamber for 3 days to extract nematodes (Barker, 1985). Nematodes were counted using an inverted light microscope (Leica DM/IRB®, Leica Microsystems, Inc.).

Mortality Comparison

In another experiment, probit regression estimates of mortality were developed and compared for conditioned and unconditioned *R. similis*. Fourteen *Anthurium andraeanum* cv. 'Mickey Mouse' were planted and inoculated as described previously. Seven plants were conditioned at 40°C for 15 minutes and the remaining 7 received of ambient drench at 24°C for 15 minutes. All plants were held for a 3-hour lag period before subsequent challenge heat treatments. At the end of the lag period, plants were separated from media, and roots were chopped into 3 to 5-cm pieces. The roots of conditioned plants were composited and mixed, and 20-g subsamples were placed in 330-µm pore bags. The process was repeated for ambient water drenched roots. Root subsamples were randomly assigned to challenge heat treatments of 45°C for 0, 2, 4, 6, and 8 minutes. Each challenge treatment was replicated 4 times and the entire experiment repeated once. Nematodes were extracted from samples within 24 hours using a mist chamber and counted using an inverted light microscope (Leica DM/IRB®,

Leica Microsystems, Inc.). The data were arcsine transformed and subjected to homogeneity of variance test and combined when appropriate. Probit regression estimates of mortality at 45°C were compared for conditioned and unconditioned nematodes.

4.4. Results

In vitro Conditioning

Mortality of nematodes between 43 and 49°C varied according to exposure times. One hundred percent mortality of *R. similis* was measured at 49°C after a 1-minute exposure. A 43°C exposure resulted in only 95% mortality after 15 minutes while the 45 and 47°C temperatures had intermediate mortality rates (Fig. 1). The *in vitro* thermal death point was investigated at 47°C, since this temperature was closest to the practical drench applications used. A probit analysis on 47°C data showed that the estimated time to kill 99.9% of nematodes ($LT_{99.9}$) was 5.1 minutes with a lower and upper 95% confidence interval of 4.7 and 5.6 minutes, respectively (Fig. 2). A Pearson χ^2 test showed no significant departure of observed data from probit regression estimates ($P = 0.58$). A 47°C for 5 minutes treatment was chosen to challenge conditioned nematodes. Data from conditioned nematodes receiving no challenge heat treatment showed some mortality resulted from 40°C and 45°C conditioning treatments (Fig. 3). However, none of the conditioned nematodes were able to survive the challenge heat treatment.

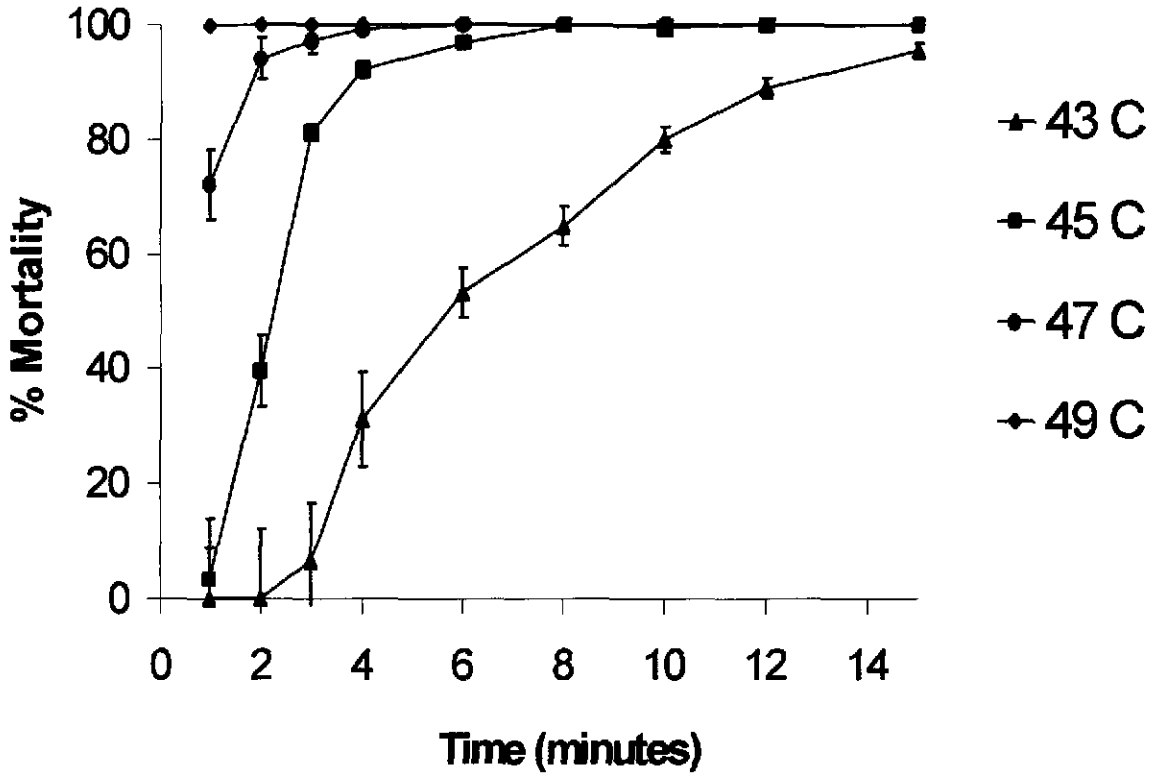

Fig. 4.1. Percent mortality of *Radopholus similis* at various temperatures *in vitro* for durations between 1 to 15 minutes. Points are mean of ten replications with standard error bars. Percent mortality was calculated by (1 – survivors/mean recovered from 0 minute treatments) x 100%.

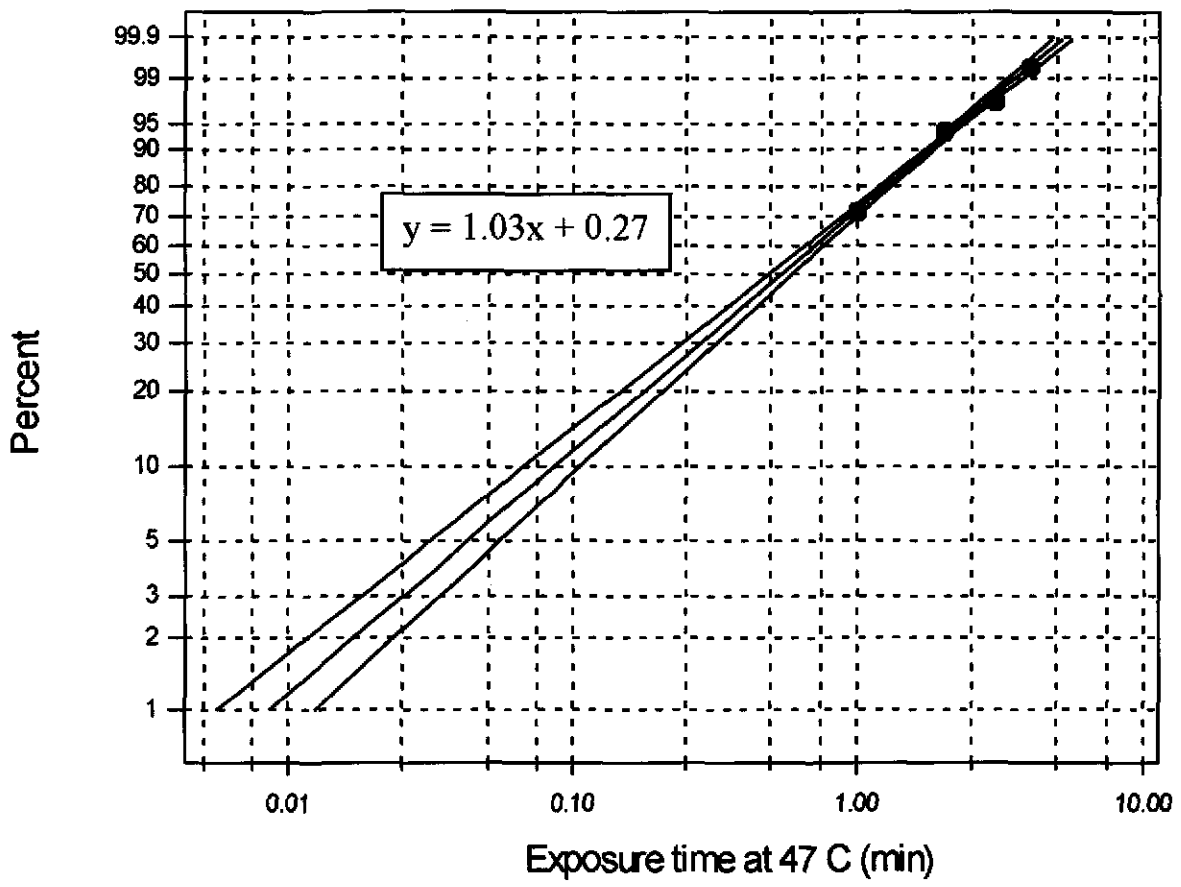

Fig. 4.2. Probit regression estimate for *in vitro* mortality of *Radopholus similis* at 47°C between 0 to 15 minutes. Points represent mean of observed data (n=10). Lower and upper 95% fiducial limits are also plotted. Linearity of exponential data is achieved by transforming time (x-axis) to ln(time) and percent mortality (y-axis) to ln(–ln(1 – % mortality). Non transformed values are plotted on axes with transformed scale for clarity.

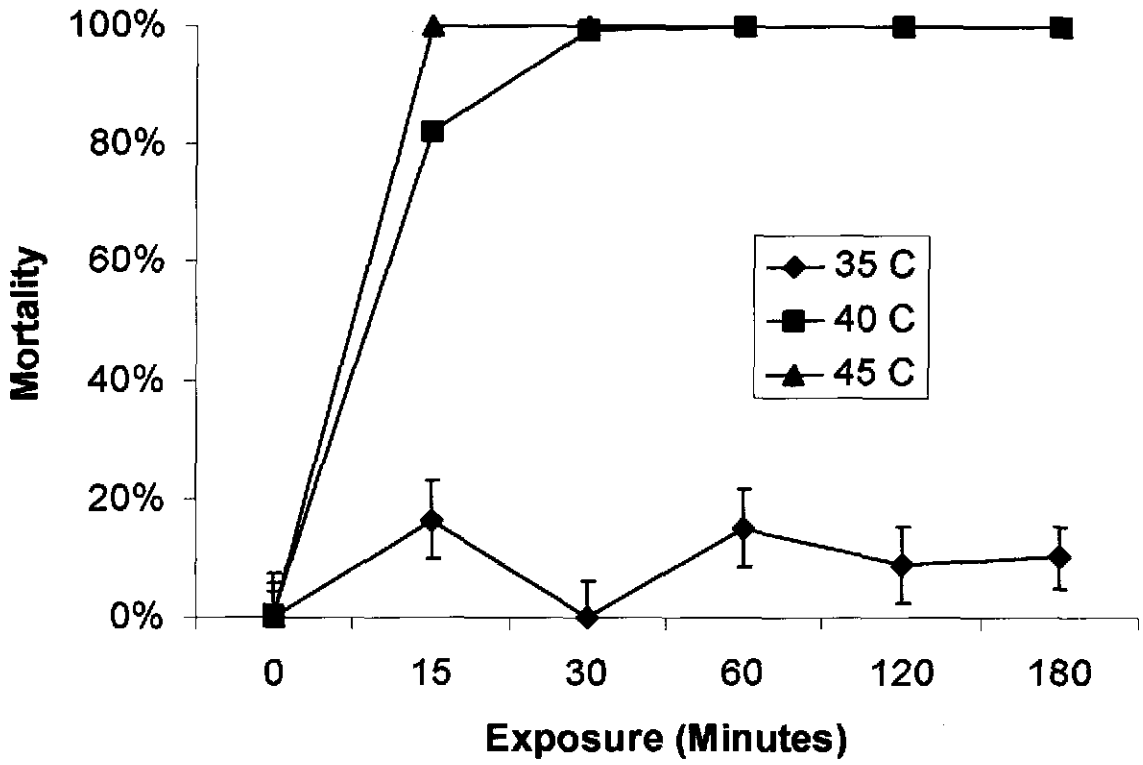

Fig. 4.3. Percent mortality of *Radopholus similis* subjected to *in vitro* conditioning treatments at 35, 40, and 45°C between 0 and 180 minutes. Each point is a mean of 8 replicates. Error bars represent standard error. Percent mortality is calculated by 1-(survivors/mean numbers of nematodes from control) x 100%.

Anthurium Conditioning

In *Anthurium*, nematodes that were conditioned for 15 minutes at 40°C prior to a standard 49°C for 12-minute treatment had low numbers of survivors in 90% of the replicates. The conditioned plants that received no challenge treatment, had a mean of 5 nematodes/g of fresh root (n=10) while plants that received the challenged treatment had means of less than 1 nematode/g of fresh root (P<0.01). Results showed that conditioning treatments alone were nonlethal.

Mortality Estimates

A test for equal slopes of probit regression estimates for survival at 45°C between conditioned and unconditioned nematodes was not significant (P = 0.85). The regression equations were nearly similar, $y = 0.11 + 1.72x$, for unconditioned nematodes and, $y = 0.15 + 1.68x$ for conditioned nematodes. Relative potency, the ratio of equally effective doses, was 1.0005. Comparison of LT_{50}, lethal time for 50% mortality was, 56 seconds for unconditioned nematodes and 55 seconds for conditioned nematodes (Fig. 4). However, Pearson χ^2 tests showed there was significant deviation between observed values and probit estimates for both regression estimates (P<0.01).

4.5. Discussion

Conditioning did not allow nematodes to develop thermotolerance against a subsequent lethal challenge heat treatment of 47°C for 5 minutes *in vitro*. Probit regression estimates of conditioned and unconditioned nematodes in *Anthurium*, challenged at 45°C, were almost identical, although the deviations from the estimates

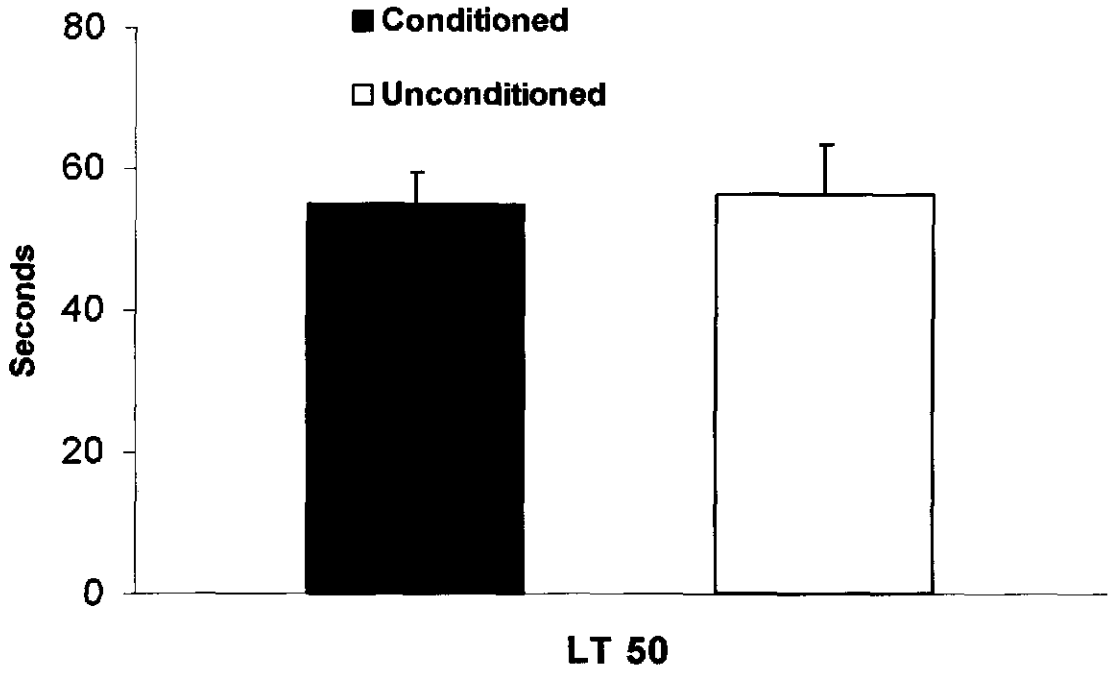

Fig. 4.4. LT_{50} probit regression estimates for *Radopholus similis* in *Anthurium* conditioned at 40°C for 15 minutes and unconditioned. Distribution of probit estimates was assumed to be loglogistic. Error bars represent the standard error.

may have been compromised by insufficient replications. Data suggest that thermotolerance does not develop from nonlethal conditioning treatments. Nonlethal conditioning applied in these experiments were designed for heat conditioning plants and do not presume to eliminate the possibility of thermotolerance arising from other conditioning regimes.

Conditioning tests in *Anthurium* indicated low numbers of survivors in conditioned plants that were subsequently drenched with 49°C water for 12 minutes. Data from sublethal hot water treatments of *Ditylenchus dipsaci* show that survivors of one heat treatment were resistant to a subsequent identical treatment and thermotolerance was indicated by a faster recovery to motile serpentine movement (Hastings *et al.*, 1952). Challenge heat treatments in our experiments were not identical and much more severe than the conditioning treatment to simulate a conditioning regime for plants followed by a control treatment for nematodes. The presence of survivors was the only indicator of thermotolerance, which can only be inferred and was not directly observed. Without further evidence, such as a protein analysis of nematodes for presence of heat-shock proteins, the presence of low numbers of survivors in conditioned plants cannot be directly attributed to thermotolerance (Selvan *et al.*, 1996).

Consideration of alternative mechanisms for the observed nematode survival is necessary. Migratory nematodes are thermotactic and migrate towards preferential temperatures (Diez and Dusenbery, 1989; Rode, 1969; Robinson, 1989). A nonlethal exposure to elevated conditioning temperatures followed by a 3-hour lag period may provide the stimulus and time necessary for the endoparasitic *R. similis* to migrate into

the stems. The challenge drench treatment was applied only to roots so nematodes that had migrated to the stem would have escaped direct exposure to target temperatures. The nematode assay 1-week later would have recovered the survivors. Until further investigation into the presence of survivors in conditioned plants, treatments to heat condition *Anthurium* should not be applied prior to hot water drenches to control *R. similis* because efficacy is seriously compromised. Investigation into conditioning treatments applied with hot air or hot water showers to avoid temperature gradients within *Anthurium* tissue, the primary stimulus for thermotaxis, should also be pursued.

4.6. Literature Cited

Barker, K.R. 1985. Sampling nematode communities. *An Advanced Treatise on Meloidogyne Vol II Methodology*. Barker, K.R., Carter, C.C., Sasser J.N. (eds). North Carolina State University Graphics: Raleigh, N.C.

Burke, J.J., J.L. Hatfield, R.R. Klein, and J.E. Mullet. 1985. Accumulation of heat shock proteins in field-grown cotton. *Plant Physiol.* 78:394-398.

Chan, H.T., Jr. and E. Linse. 1989. Conditioning cucumbers for quarantine heat treatments. *HortScience*. 24(6):985-989.

Diez, J.A. and D.B. Dusenbery. 1989. Preferred temperature of *Meloidogyne incognita*. *Journal of Nematology*. 21:99-104.

Hastings, R.J., J.E. Bosher, and W.M. Newton. 1952. The revival of the narcissus bulb eelworm *Ditylenchus dipsaci* (Kuhn) Filipjev, from sublethal hot water treatments. *Scientific Agriculture*. 32:333-336.

Ko, M.P. D.P. Schmitt, and B.S. Sipes. 1996. Axenizing and culturing endomigratory plant-parasitic nematodes using Pluronic F127, including its effects on population dynamics of *Pratylenchus penetrans*. *J. of Nematology*. 28(1):115-123.

Robinson, A.F. 1989. Thermotactic adaptation in two foliar and two root-parasitic nematodes. *Revue de Nématologie*. 12:125-131.

Rode, H. 1969. On the behaviour and sensitivity of potato-root eelworm larvae to thermal gradient stimuli within the superoptimal temperature zone. *Nematologica*. 15:523.

Selvan, S., P.S. Grewal, T. Leustek, and R. Gaugler. 1996. Heat shock enhances thermotolerance of infective juvenile insect-parasitic nematodes *Heterorhabditis bacteriophora* (Rhabditida: Heterorhabditidae). *Experientia.* 52:727-730.

Snutch, T.P. and D.L. Baillie. 1983. Alterations in the pattern of gene expression following heat shock in the nematode *Caenorhabditis elegans. Canadian Journal of Biochemistry and Cell Biology.* 61:480-487.

Tsang, M.M.C., Hara, A.H., Sipes B.S. 2001. Hot Water Drenching System Disinfesting Roots and Media of Potted Plants of Burrowing Nematodes. *Applied Engineering in Agriculture.* 17(4): 533-538

Woolf, A.B. and M. Lay-Yee. 1997. Pretreatments at 38°C of 'Hass' Avocado confer thermotolerance to 50°C hot water treatments. *HortScience.* 32:705-708.

Yarwood, C.E. 1967. Adaptation of Plants and Plant Pathogens to Heat. *Molecular Mechanisms of Temperature Adaptation.* Prosser, C.L. (ed.), Am. Assoc. for the Advancement of Science: Washington D.C. pp. 75-89.