ERADICATION OF *ROTYLENCHULUS RENIFORMIS* FROM A VOLCANIC CINDER MEDIUM USING STEAM STERILIZATION

R. Y. M. Cabos[1]*, M. M. C. Tsang[2], A. H. Hara[3], and A. Kawabata[3]

[1]USDA, Agricultural Research Service, U.S. Pacific Basin Agricultural Research Center, 64 Nowelo Street, Hilo, Hawaii 96720, USA. [2]University of Hawaii at Hilo, College of Agriculture, Forestry and Natural Resource Management, 200 W. Kawili St., Hilo, HI 96720, USA. [3]University of Hawaii at Manoa, College of Tropical Agriculture and Human Resources, 875 Komohana St., Hilo, HI 96720, USA. *Corresponding author: roxana.cabos@ars.usda.gov

ABSTRACT

Cabos, R. Y. M., M. M. C. Tsang, A. H. Hara, and A. Kawabata. 2012. Eradication of *Rotylenchulus reniformis* from a volcanic cinder medium using steam sterilization. Nematropica 42:245-252.

In Hawaii, reniform nematode, *Rotylenchulus reniformis,* damages many agricultural crops and disrupts plant trade because of the regulations imposed against this pest by the state of California and other national and international markets. Nurseries in Hawaii must follow strict certification requirements to export potted plants to these markets where *R. reniformis* is not established. Mined volcanic cinder, utilized as a potting medium, may contain reniform nematode and needs to be disinfested for use by nematode certified nurseries. Two steam treatment systems of different capacities were evaluated for their efficacy to disinfest the cinder of *R. reniformis*. The low capacity system consisted of a portable steam generator connected to a steam cart with a media volume of 1.68 m^3. The large capacity system consisted of a dump truck bed modified with an intake manifold and steam distribution pipes connected to a large capacity steam generating boiler to sterilize a 24.5 m^3 load of media. Packets of nematode-infested cinder containing *R. reniformis*-infested roots of *Ipomoea batatas* were buried in various locations and different depths in the cinder contained in the two steaming systems. Temperature probes were placed inside or adjacent to the packets and observed during the sterilization process. The steam did not penetrate and distribute in the medium at a consistent rate in both steaming systems resulting in cold spots. These were identified and monitored to ensure that treatment time was sufficient to reach target temperature throughout the medium mass. Once the steam was evenly distributed both systems were successful at eradicating all live *R. reniformis.*

Key words: certified potting medium, nematode certification, ornamental industry, plant trade, regulatory, reniform nematode.

RESUMEN

Cabos, R. Y. M. , M. M. C. Tsang, A. H. Hara, and A. Kawabata. 2012. Erradicación de *Rotylenchulus reniformis* de medio de ceniza volcánica utilizando esterilización con vapor. Nematropica 42:245-252.

En Hawaii, el nematodo reniforme, *Rotylenchulus reniformis*, causa daños a muchos cultivos agrícolas e interfiere con el comercio de plantas debido a las regulaciones impuestas contra el movimiento de esta plaga en el estado de California y otros mercados nacionales e internacionales. Los viveros en Hawaii deben seguir estrictos requerimientos de certificación para exportar plantas en macetas a lugares en donde *R. reniformis* no está establecido. La ceniza volcánica, usada como medio de cultivo, puede contener nematodo reniforme y debe desinfestarse antes de ser usada en viveros libres de nematodos. Se evaluó la eficacia para desinfestar ceniza en dos sistemas de tratamiento con vapor de diferentes capacidades. El sistema de baja capacidad consistió de un generador de vapor portátil conectado a un vagón con capacidad de 1.68 m^3 de medio de cultivo. El sistema de alta capacidad consistió de una volqueta modificada y tubería para distribución del vapor conectada a un sistema generador de vapor con capacidad para una carga de 24.5 m^3 de medio. Se enterraron varios paquetes de ceniza infestados con *R. reniformis* y conteniendo raíces de *Ipomoea batatas* infectadas con el nematodo en distintos lugares y a diferentes profundidades en los dos sistemas evaluados. Se colocaron termómetros junto a los paquetes o dentro de ellos para obtener medidas de la temperatura durante el proceso de esterilización. El vapor no penetró y se distribuyó en el medio de manera uniforme, resultando en sitios fríos. Se identificaron estos sitios y se extendió el tiempo de tratamiento para asegurar que se alcanzara la temperatura necesaria en todo el volumen de medio. Una vez se logró distribuir el vapor de manera uniforme, ambos sistemas eliminaron todas las formas vivas de *R. reniformis* de manera exitosa.

Palabras clave: certificación para nematodos, comercio de plantas, industria de ornamentales, nematodo reniforme, medio de cultivo certificado, regulación.

INTRODUCTION

In Hawaii, the reniform nematode, *Rotylenchulus reniformis*, damages many agricultural crops and disrupts plant trade because of the regulations imposed against this pest by the state of California and other national and international markets (Chitambar, 2007). Foliage and landscape nurseries that export potted plants to these markets must be nematode certified. Hawaii Department of Agriculture (HDOA), Plant Quarantine Branch, implements burrowing (*Radopholus similis*) and reniform nematode certification programs under the California-Hawaii master permit QC 650 (Anonymous, 2011). Detection of these regulated nematodes in the nurseries by HDOA inspectors causes the loss of the nematode certification and requires expensive sanitation procedures by the growers in order to have the certification reinstated. Furthermore, the interception of regulated nematodes in plant shipments by California Department of Food and Agriculture (CDFA) officials has more serious consequences for the growers and results in rejection of the shipments and heavy financial losses.

Nurseries in Hawaii utilize lava rock fragments, called cinders, as a potting medium. Volcanic cinders are glassy and contain numerous gas bubbles "frozen" into place as magma exploded into the air and then cooled quickly. These small volcanic pieces are harvested from cinder cones which eventually become pits due to the mining. Cinders in recently formed cinder cones are free from vegetation and consequently plant-parasitic nematodes. However, older cinder mines are surrounded by soil and weeds that are hosts of reniform nematode with consequent nematode contamination of the mined cinder. This nematode-contaminated cinder is not a suitable potting medium to be used in certified nurseries. Growers must steam pasteurize nematode-contaminated cinders at temperatures of 71°C – 93°C at the center of the media for 30 minutes in order to free the volcanic pieces from plant-parasitic nematodes. After disinfestation, cinder is stored in above ground bins or on a concrete pad with impermeable 46 cm-high siding to prevent possible contamination (Anonymous, 2011). Steam sterilization is currently the only approved treatment required by the state of California under the Master Permit QC 650 to disinfest this potting medium. The use of chemicals, like Vapam, is not permitted (Anonymous, 2011).

The use of steam to disinfest soil is a technique which has been in practice for over 100 years (Newhall, 1955). In addition to eradicating plant-parasitic nematodes, steam also rids the media of most soil-borne pathogens and weeds (Baker, 1962). Other benefits of steam include the lack of toxic residues after treatment and the low potential for organisms to develop resistance to this control method. Steam is produced by the evaporation of water heated by a boiler. It travels through the media as water vapor until it condenses on particles of soil. The distribution of heat through the media is more efficient with steam than dry heat or hot water (Baker, 1962). To be effective at soil disinfestation, the temperature of the media must reach the thermal lethal point of the colonizing pest.

In this research, two steam treatment systems of different capacity were evaluated for their efficacy at disinfesting volcanic cinder potting medium from *R. reniformis*.

MATERIALS AND METHODS

Thermal Lethal Point

The thermal lethal point of *R. reniformis* was determined by developing a kill curve by subjecting reniform nematodes to increasing amounts of heat. *Rotylenchulus reniformis* were obtained from inoculated papaya, *Carica papaya*, plants raised in 15.5-cm-diameter clay pots containing 1:1 steam sterilized soil:silica sand mix in a greenhouse in Hilo, Hawaii. Soil from the pots was placed on Baermann funnels overnight and live vermiform nematodes were collected through a 20 µm pore size diameter mesh sieve 24 and 48 hours later (Walker and Wilson, 1960). Immediately following the extraction, active reniform nematodes of mixed life stages (juveniles, males, and immature females) were placed in 1 mL of water in 1.5 mL microcentrifuge tubes. Each tube contained approximately 40 – 50 nematodes and each time/temperature treatment was replicated 3 times. Temperatures tested were 47°C, 48°C, 49°C, 50°C, and a control at an ambient temperature of 25°C. Each temperature was tested at 2, 4, 6, 8, 10, 12, and 14 minutes with the exception of 50°C which was tested at 1, 2, 4, and 6 minutes. Tubes were placed in a dry bath incubator with thermocouple probes inside the nematode suspension. Measurement of time intervals began when the water suspension within the tube reached the target temperature according to a 12-Channel Digi-Sense® Thermocouple Scanning Thermometer model 01X339303. At the end of the treatment time, tubes were immediately transferred to a cold water bath until ambient temperature was reached. Nematode mortality was measured after 24 hours by observing living nematodes at 50x magnification using a Leica inverted microscope. Nematodes that failed to respond after probing were considered dead. The experiment was repeated using a circulating hot water bath in place of a dry bath incubator. Abbott's formula was used to calculate the corrected mortality rate (Abbott, 1925).

Small Scale Steam Sterilization

Trial 1. The trial was conducted at the University of Hawaii-Hilo College of Agriculture, Forestry and Natural Resources' farm, located in the Panaewa Ag lots near Hilo, Hawaii. A steaming cart holding 1.68 m^3 of 1.59 cm size cinder double screened was used for the treatment (Fig. 1). Steam was generated using a

portable Sioux Steam Flo steam generator SF-11 (175 kg steam/hour) and delivered into a plenum, the top of which served as the cart bed (3 m L x 0.5 m H x 1.2 m W) via an intake manifold pipe. The bed was perforated with 0.63 cm holes drilled at 7.6 cm intervals.

Rotylenchulus reniformis inoculum was obtained from soil and roots of papaya grown in a greenhouse in Hilo, Hawaii and from soil and roots of sweet potato, *Ipomoea batatas*, from a field plot in Pepeekeo, Hawaii. Eggs were recovered from sweet potato roots using the NaOCL method (Hussey and Barker, 1973). The obtained suspension was left stirring at room temperature for 48 hours. *Rotylenchulus reniformis* juveniles, males, and immature females were collected from 100 cm^3 aliquots of infested soil using the Baermann funnel method (Walker and Wilson, 1960).

In order to verify the effect of the steaming treatments on the reniform nematode's survival, a series of cinder packets were prepared. The cinder packets consisted of *R. reniformis*-infested *I. batata* roots covered by 1200 cm3 of cinder (3.81 cm minus) infested with 2,160 mixed life-stages of *R. reniformis* and wrapped in non-wire mesh screens (Fig. 2). A variety of mesh screens with different pore sizes was selected to minimize interference from the pore size in the diffusion of steam in the cinder packets. For this purpose, large pore mesh screens were used for packets 1 – 3 (pore size = 2.01 x 3.18 mm), packets 4 – 6 and 13 (pore size = 1.81 x 3.06 mm), and packets 7, 8, and 14 (pore size = 2.4 x 2.24 mm). Small (25 x 50 µm) pore mesh insect screen was used for packets 10 – 12 and 15. Each cinder packet was assembled by laying a 30.5 cm x 30.5 cm piece of the selected mesh screen in a plastic tub and adding 600 cm^3 of cinder. A volume of ten mL of nematode inoculum, consisting of 617 eggs and vermiform nematodes kept in a stirred 490 mL water suspension, was dispensed with a pipette over the cinder layer. Five grams of *I. batatas* roots from a field plot infested with *R. reniformis* were wrapped in two task wipers and placed on top of the cinder. A 10 mL aliquot of the inoculum (617 *R. reniformis*) was dispensed on the wrapped roots. A uniquely colored piece of flagging tape was placed next to the roots to assist in later identifying the packet number. Roots were covered with 600 cm^3 of cinder and a final aliquot of 15 mL of inoculum (926 *R. reniformis*) was dispensed randomly over the top layer of cinder. The mesh screen was weaved closed with 18 gauge steel wire which held an imprinted metal tag containing the packet number.

In addition to the cinder packets, nematode-inoculated tubers of *I. batatas* were placed in the steaming cart. A deep hole was opened in the tubers by removing a root tissue core with a No. 6 cork borer (0.9 mm diameter). A nematode suspension of 250 *R. reniformis* was pipetted inside the drilled hole, which was closed with the removed core and fastened with a wire to keep the nematode suspension in place.

The treatment target temperature was 71°C for 30 minutes. A 12-channel scanning thermometer

Fig. 1. Soil cart used for small scale steam sterilization of a volcanic cinder medium.

Fig. 2. Inserting thermocouple probes into *Rotylenchulus reniformis* inoculated large pore mesh cinder packet.

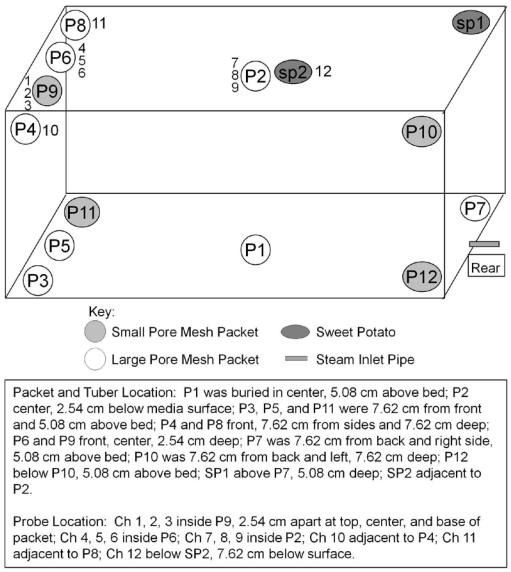

Fig. 3. Drawing of a soil cart used during small scale steam sterilization of a volcanic cinder medium for trial 1. Note locations and depths of thermocouple probes (Ch 1–12) and *Rotylenchulus reniformis* inoculated small or large pore mesh cinder packets and sweet potato tubers (SP). Packets 13-15 were used as positive controls that were not exposed to steam treatment (not illustrated in Fig. 3).

(Digi-Sense®, Cole Parmer, IL) was used to record temperature readings throughout the trial. Cinder packets and sweet potato tubers were placed in the trailer at various locations and depths (Fig. 3). Thermocouples were inserted in three (P2, P6, and P9) of the cinder packets to monitor the temperature during steam treatment (Fig. 2). Arrangements and depth of packets and probes are shown in Figure 2. Packets 13, 14, and 15 were not exposed to the steam treatment (not illustrated in Fig. 3).

After the steam treatment, inoculated cinder packets and tubers were retrieved from the soil cart. A subsample of 100 cm^3 of cinder was removed from each packet, wrapped in two delicate task wipers, placed in a Baermann funnel, and covered with water. After 24 hours, water from each funnel was drained and poured through a 20 µm pore size diameter mesh sieve. Sieves were rinsed and nematodes were collected in 40 mL of water. The solution was poured into 50 mL tubes and centrifuged for 4 minutes at 420 g. Supernatant was removed as the pellet and 5 mL of remaining water were conserved. The solution was mixed and poured into a counting dish. The entire dish was observed for living nematodes under 50x magnification with a Leica inverted microscope. Water in the Baermann funnels was replaced, held for an additional 24 hours, and the extraction repeated. Two additional subsamples for a total of 200 cm^3 from each cinder packet were processed with the same procedures and following rigorous sanitation practices. The remainder (900 cm^3) of each of the cinder packets was stored in a sealed 7.6 L plastic bag until all of the contents could be processed. Control packets were processed after the steam-treated packets.

Bundles of sweet potato roots were removed from the cinder packets and processed using the bag extraction method. Roots including the surrounding task wipers were submerged ¾ high with water in a 250 mL beaker. After 48 hours the resulting nematode suspension in water was poured through a 150 µm and 20 µm mesh sieve. Nematodes were collected in 40 mL of water and reduced to 5 mL following the centrifugation procedure described above. This operation was repeated after soaking the root bundles for an additional 2 days. The *I. batatas* tubers were also subjected to the bag extraction method after the plugs were removed.

The remaining 900 cm^3 of each cinder packet left inside the 7.6 L plastic bags was suspended in water in the same bags and incubated for 72 hours to allow juvenile hatching from eggs. The water suspension of nematodes and debris after stirring and flushing the cinder was poured through 150 µm and 20 µm nested sieves and concentrated in 40 mL of suspension. Nematodes were separated from the debris by the sugar flotation technique (Jenkins, 1964) and observed using an inverted microscope. This operation was repeated twice.

Trial 2. Nematode inoculum was obtained and extracted as previously described. Only eight inoculated cinder packets were used for the experiment. Packets 1 – 4 and 7 were wrapped with large (1.81 x 3.06 mm) pore mesh screens, and packets 5, 6, and 8 with small (25 x 50 µm) pore mesh insect screens. Packets were assembled as previously described except each packet was inoculated with a 40 mL suspension containing 1,755 *R. reniformis* of mixed life stages. *Ipomea batatas* tubers were cored and inoculated with a 4 mL suspension containing approximately 175 *R. reniformis* (vermiform and eggs).

Inoculated cinder packets were all placed 2.54 cm below the surface of the cinder (Fig. 4). Arrangement and depth of probes are shown in Figure 4. Nematode extraction was carried out after the steam treatment as previously described.

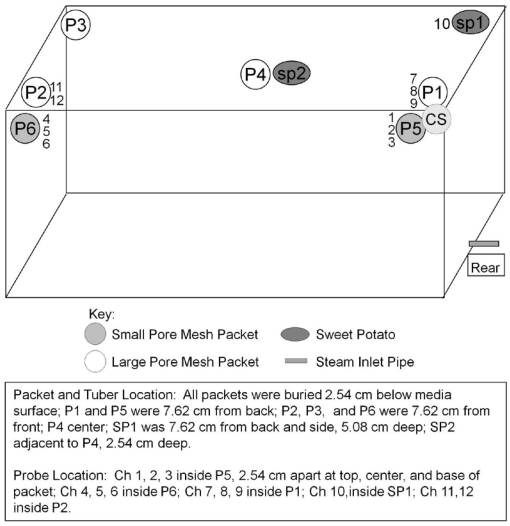

Fig. 4. Drawing of a soil cart used during small scale steam sterilization of a volcanic cinder medium for trial 2. Note locations and depths of thermocouple probes (Ch 1–12) and *Rotylenchulus reniformis* inoculated small or large pore mesh cinder packets and sweet potato tubers (SP). CS (coldest spot) indicates the location of the last probe to reach target temperature. Packets 7-8 were used as positive controls that were not exposed to steam treatment (not illustrated in Fig. 4).

Large Scale Steam Sterilization

The steam treatment was conducted at Sanford's Service Center near the town of Pahoa, Hawaii, using a large capacity steam generating boiler (Saskatoon Boiler, 986 kg steam per hour). A modified steam delivery system was installed in the truck bed (5.8 m L x 1.5 m H x 2.3 m W) to sterilize the media (Fig. 5). An intake manifold was placed above the tailgate and connected to a mainline pipe (5.08 cm diameter). Four separate laterals (5.08 cm diam.) ran down the tailgate

to four pipes lying on the floor of the bed. The pipes on the bed floor were 15.24 cm in diameter, cut in half and drilled with 1.27 cm diameter holes with 15.24 cm spacing to release the steam. A 6.1 m hose (5.08 cm diam.) connected the boiler to the intake manifold. The bed of the truck was raised approximately 10 degrees to drain accumulating water.

Nematode inoculum was obtained from soil and roots of *I. batatas* collected from a field plot in Pepeekeo, Hawaii. Inoculum was processed as described above. Packets were the same as those in the previous trials, but were inoculated with 580 mixed life stages of *R. reniformis*/packet and each contained 10g of infected *I. batatas* roots. Only small (25 x 50 µm) pore mesh screens were used to wrap the packets.

The treatment target temperature was monitored with a 12-Channel scanning thermometer as previously described. Additional hand-held temperature probes and an infrared thermometer measured instantaneous readings periodically during the treatment. Prior tests have shown that the steam heats the base of the media in the center of the bed, then radiates along the sides as it rises to the surface, moving toward the center. Thermocouple probes were placed strategically in the bed to monitor heating (Fig. 6) as 24.5 m*3* of cinder (3.81 cm minus) was incrementally loaded into the bed of the dump truck at the cinder pit site (Fig. 5). Packets of inoculated cinder were placed adjacent to the 12 probes. Arrangement and depth of probes and packets are shown in Figure 6. Packets 13 and 14 were used as positive controls and were not exposed to the steam treatment (not illustrated in Fig. 6).

The truck was moved to the base yard where the probes were attached to the scanning thermometer unit and the bed covered with a canvas tarp (4.9 m L x 3.1 m W).

The protocol for extracting *R. reniformis* from the cinder packets after the steam treatment was the same as previously described.

RESULTS

Thermal Lethal Point

Nematode mortality increased with increased temperature and exposure time (Fig. 7). Ninety-nine percent of reniform nematodes were killed after 12 and 14 minutes at 47°C. One hundred percent mortality was achieved after 10 minutes at 48°C, 2 minutes at 49°C, and 1 minute at 50°C.

Results differed slightly when the nematode suspension was exposed to heat in a dry bath incubator as compared in a circulating hot water bath. Gaps existed between the wells of the incubator and the microcentrifuge tubes likely resulting in a non-uniform distribution of heat within the tube. The mortality curve was based solely on results obtained from the circulating hot water bath.

Fig. 5. Modified steam-delivery system in dump truck bed at the volcanic cinder mining site.

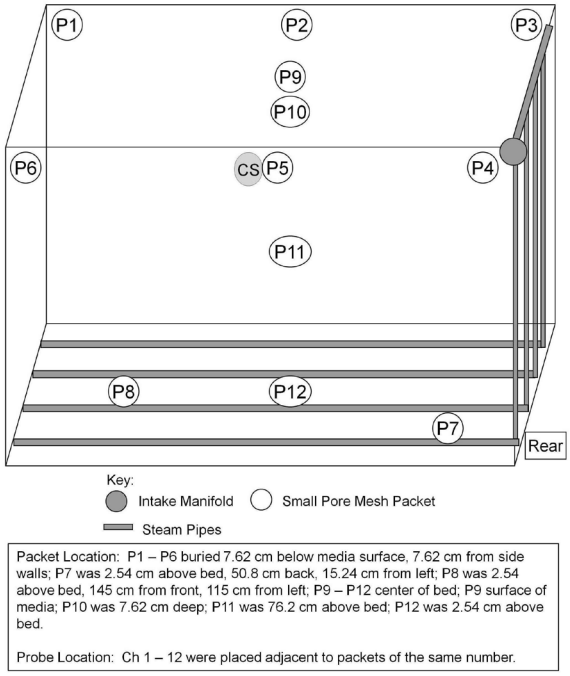

Fig. 6. Drawing of the modified truck bed used during large scale steam sterilization of a volcanic cinder medium. Note locations and depths of thermocouple probes (Ch 1-12) and *Rotylenchulus reniformis* inoculated cinder packets adjacent to probes. CS (coldest spot) indicates the location of the last probe to reach target temperature. Packets 13 and 14 were used as positive controls that were not exposed to steam treatment (not illustrated in Fig. 6).

Small Scale Steam Sterilization

Trial 1. Thermocouple probes (Ch 10-11) located in the front of the soil cart were the first to reach 99°C (Fig. 8A). Within 55 minutes, probes 11 and 10 attained the maximum temperature. Probes (Ch 4-6) inside the open mesh cinder packet 6 registered 99°C at

75 minutes. Ten minutes later probes (Ch 1-3) within the fine mesh packet adjacent to it also attained 99°C. Eighty-five minutes after the steam sterilization was initiated, the remaining probes (Ch 7-9, 12) reached 99°C. Five minutes later the steam generator was shut down and temperatures continued to be recorded. The target temperature of 71°C was exceeded for the next 55 minutes while the cart remained covered.

Seven live *R. reniformis* juveniles were recovered from inoculated cinder packet 10. No thermocouple probes were monitoring the temperature inside or adjacent to the packet. Live reniform nematodes were not recovered from the other treated packets or sweet potato tubers. Extractions from untreated cinder packets (13-15) contained 39, 45, and 27 live *R. reniformis*, respectively, whereas 4 *R. reniformis* were recovered from the untreated tuber.

Trial 2. Similar to the first trial, the media in the front of the soil cart was the first to reach target temperature (Fig. 8B). Probes inside the cinder packets 2 (Ch 11-12) and 6 (Ch 4-6) reached 99°C within 115 minutes. The size of the mesh made little difference on the ability of steam to penetrate the cinder and roots inside. At 145 minutes some probes (Ch 1, 7-9) located in the rear of the cart began to register at 99°C. The remaining thermocouple probes (Ch 2-3, 10) attained 99°C at 160 minutes. These probes were located in fine mesh packet 5 (Ch 2-3) at the left rear of the cart and inside the sweet potato tuber in the right rear of the cart (Ch 10).

No live *R. reniformis* were extracted from inoculated cinder packets and sweet potato tubers that were subjected to the steam treatment. Thirty-four live *R. reniformis* were recovered from an untreated tuber and 79 and 49 *R. reniformis* were extracted from the two untreated cinder packets.

Large Scale Steam Sterilization

Initial temperature readings from the 12-channel scanner ranged from 20°C to 23°C (Fig. 9). Within ten minutes thermocouple probe 8, on the floor bed, registered 99°C followed by probe 7 and 12, five and fifteen minutes later respectively. Probe 11 located in the middle of the media, 76.2 cm above the floor bed, attained the maximum temperature within eighty minutes after initiating the steam treatment. Thermocouple probes along the bed walls 7.62 cm below the media surface (Ch 1-4, 6) had all reached 99°C within one hundred and fifty minutes with the exception of probe 5. Probe 9 situated on top of the media surface and not immersed in the cinder reached 73°C exceeding the target temperature after one hundred and eighty-five minutes but never attained 99°C. The probe (Ch 10) 7.62 cm below it registered 99°C twenty minutes later. The coldest spot (CS) in the truck bed was located adjacent to probe 5, 7.62 cm deep along the center of the left wall (Fig. 6). The probe (Ch 5) continued to register at ambient temperature until

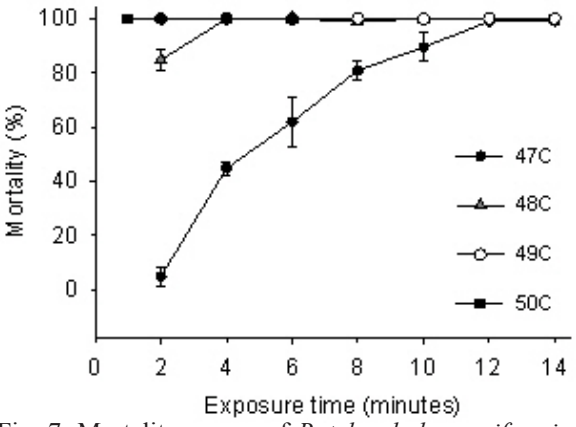

Fig. 7. Mortality curves of *Rotylenchulus reniformis* in aqueous suspensions heated in a circulating hot water bath. Abbot's formula was used to calculate the corrected mortality rate. Bars represent the standard error of the mean.

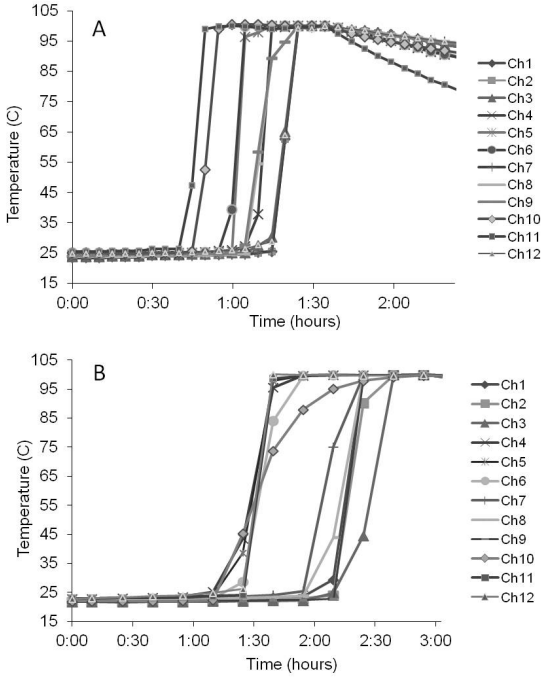

Fig. 8. Temperature rise values registered by 12 thermocouple probes during steam sterilization of a volcanic cinder medium in a soil cart during trial 1 (A) and 2 (B). Probes were placed inside cinder packets inoculated with *Rotylenchulus reniformis* and in various locations throughout the cart.

two hundred and five minutes after the treatment began followed by a rapid rise to 99°C. At that time, the thirty minute sterilization exposure commenced and ten minutes later the boiler was turned off. Probe 9 located

on the media surface dropped to 81°C momentarily. All probes continued to register above the target temperature of 71°C when monitoring of probes was terminated after the required exposure time. Steam was still observed rising from the volcanic cinder medium two hours following the sterilization treatment.

No live reniform nematodes were recovered from any of the steam treated cinder packets and sweet potato roots. Extractions from the non-treated packets yielded 27 and 43 live *R. reniformis*.

DISCUSSION

Complete mortality occurred in aqueous suspensions of *R. reniformis* at 48°C within 10 minutes during laboratory experiments. Heald and Robinson (1987) observed a 90% mortality rate when soil was heated to 47°C for less than an hour. This is well below the Department of Agriculture's temperature requirement of 70°C for sterilizing media used in potted exports. The limiting factor for effective soil disinfestation appears to be the ability of the steam to effectively penetrate all areas of the media. A margin of error must be incorporated due to soil heterogeneity (van Loenen *et al.*, 2003). After comparing steam sterilization in four soil types, Minuto *et al.* (2005) concluded that diffusion of steam likely occurred by isothermal fronts running parallel to the soil surface and was not affected by soil composition, texture, or heterogeneity of the mixture.

In this research, the steam did not penetrate and distribute in the media at a consistent rate or pattern, thus resulting in a non-uniform heating front advancing from the bottom to the top. This was observed in both the modified dump truck and soil cart and is probably due to the limitations in the design of the particular steam supply systems. Areas that are the last to reach the target temperature (cold spots) must be identified and monitored for each individual system, and the duration of treatment time must be adequate to ensure complete kill of the targeted pathogen. The coldest spot in the soil cart was located towards the rear at the steam inlet port (Fig. 4) where live *R. reniformis* juveniles were recovered from inoculated cinder packet 10 in trial 1 (Fig. 3). Because most of the thermocouple probes in that trial were located towards the front end of the cart (Fig. 3), steam flow was cut too early (90 minutes into the treatment) based on the temperature readings, thus the inoculated media in packet 10 probably did not reach target temperature even with the 55 minute post treatment waiting time before the samples were recovered. In contrast to trial 2 where the thermocouple probes 1, 2, 3, 7, 8, and 9 were located in the cold spot region, no survivors were recovered in packet 5 (Fig. 4) as the steam supply was maintained for a longer period (160 minutes) based on the temperature readings. The cold spot in the dump truck was located near the top midway along the left wall and required about three and a half hours after initiating the treatment to reach target temperature (Fig. 6).

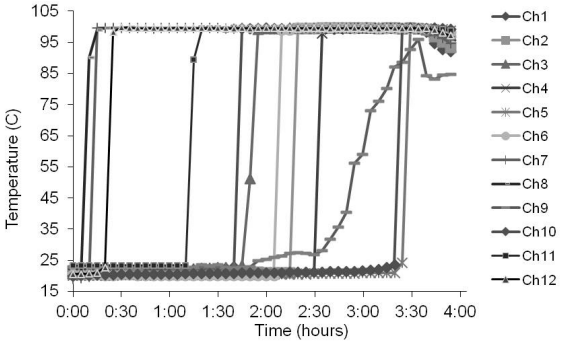

Fig. 9. Temperature rise values registered by 12 thermocouple probes during steam sterilization of a volcanic cinder medium in a modified truck bed. Probes were placed adjacent to cinder packets inoculated with *Rotylenchulus reniformis*.

With rising fuel prices, steam treatments add to the production costs of nurseries. The treatment must also be completely effective against the targeted pest to avoid loss of certification by affected growers. More efficient steaming techniques must be investigated to reduce both total treatment time and variability in different systems as observed in this study. Perhaps a negative pressure system can be explored where steam is injected on the top under the canvas cover and is pulled downward into the media by suction pipes installed at the bottom. The hot water from condensing steam can then be advantageously used to preheat the cinder in front of the advancing steam. A dump truck because of its exposed metallic construction is not an ideal heat treatment container but is very practical for industry application as large volumes of cinder can be conveniently sterilized and transported on demand to nurseries. This also avoids the need to build an expensive stationary steaming chamber, and saves on additional loading and handling costs. Also, the tests in this research were done outdoors unprotected from breeze and wind. It is difficult to insulate the metal walls of the dump truck or soil cart due to the abrasive nature of cinder and the normal working environment of these pieces of equipment. Heat loss from the exposed metal can be reduced if the steaming is carried out between protected permanent or temporary walls.

Sterilizing potting media can have both beneficial and adverse effects on plant growth. The obvious beneficial effect in this study is cleaning the media of *R. reniformis* in addition to other pathogens and weed seeds. Malowany and Newton (1947) found an increase in water soluble phosphorus after steam sterilization although the differences were greater in soil with higher organic matter contents. However avocado seedlings planted in steam sterilized soil demonstrated a phosphorus deficiency in the leaves (Martin *et al.*,

1973). Although levels in the soil were adequate, it was speculated that the destruction of mycorrhizal fungi reduced the plant's ability to uptake this nutrient. Local nursery operators using steamed cinder reported a reduction in root rot incidence and good plant growth in potted dracaena (Andrew Kawabata, personal communication). Additional research is needed to detect other impacts of steam sterilization treatments on potted ornamentals in Hawaii.

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