THE DEVELOPMENT AND TRANSFER TO END USER OF A METHYL BROMIDE ALTERNATIVE TREATMENT TO DECONTAMINATE ITEMS INFESTED WITH THE GOLDEN NEMATODE

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The golden nematode is an introduced pest of potatoes that is under state and federal quarantine. A treatment is required to decontaminate equipment and other items when they are moved from golden nematode infested areas to non-infested areas. Since regulations were imposed in 1944, methyl bromide has been the treatment of choice to disinfest items to free them of the golden nematode. Because methyl bromide was so effective, there were no efforts to develop alternative treatments for this program. In 1944, research was initiated on alternative treatments to replace methyl bromide in the golden nematode program. Heat was identified as a potential treatment for decontaminating items infested with the golden nematode but dehydrated golden nematode cysts were relatively insensitive to heat. Steam was chosen as a source of heat as it provided sufficient moisture to sensitize the cysts to heat and sufficient temperatures necessary to kill the eggs inside the cysts. Several tests demonstrated that steam treatment of 60ºC for one hour was lethal to golden nematode eggs consistently enough to be used in a quarantine setting.

To determine if this treatment could be effectively implemented at the field level, USDA, APHIS Methods Development specialists worked with Sioux Steam Cleaner Corp., Beresford, South Dakota to develop the steam generating equipment necessary to achieve the treatment protocol (60ºC for one hour) and the enclosures needed to contain the steam. After demonstrating the equipment was able to sustain the target temperatures, final tests were conducted in Avoca, NY.

A weather resistant canvas tent 8’ high x 10’ wide x 20’ long with a 10’ peak (1600 ft^3) was utilized in this test. Recording devices to monitor temperatures under the tent, were two recording thermometers with 4 thermocouples each, to provide instant readings and a Data Trace system with 16 remote tracers. Thermocouples and remote probes were situated along the sides, back and front of the tent and equipment to provide a thermal map of the enclosure and equipment. Equipment used for treatment was a Ford 2600 tractor with an attached hitch with 3 soil sampler wheels. Packets of golden nematode cysts, ten with and without soil, were placed in various locations on the tractor and soil sampler to simulate golden nematode infestations. Sixteen remote probes and 8 thermocouples attached to a hand held Omega printing thermometer (to visually monitor temperatures) were placed on the equipment inside the tent enclosure and along side the packets of cysts. Temperatures were recorded at two-minute intervals. Ambient and equipment temperatures ranged from 2ºC to 4ºC at the onset of the treatment. Timing of the treatment did not commence until all temperature probes reached target temperature (60ºC). To maintain a minimum temperature of 60ºC on the equipment surface, the ambient temperature inside the tent was allowed to reach 71ºC. The steamer was switched off at 71ºC and when the temperature fell to 68ºC it was switched on again. Temperatures of 60ºC or greater were maintained on the equipment surface for 60 minutes (Table 1). At the conclusion of the test, the steam was evacuated by unzipping the front flap of the tent and the cysts were removed. This test was repeated once.

Assay procedures to determine the effectiveness of the treatment consisted of subjecting cysts to hatching tests and the inoculation of susceptible potato plants with hatched juveniles and the unhatched eggs that remained after the hatching test. To induce hatching, the cysts are soaked in water for 5 days and then placed in potato root diffusate (PRD) in ELISA plates. The number of juveniles that emerge were counted weekly and fresh PRD added. After three weeks of hatching, the cysts were crushed and the number of viable and nonviable eggs remaining determined. Potato plants that had been inoculated with hatched juveniles and/or unhatched eggs were examined 12 weeks after inoculation for nematode reproduction.

Temperatures of 60ºC or greater were maintained on the equipment surface for 60 minutes (Table 1). Temperatures inside the tent reached target temperature in about 30 minutes. Even in adverse weather, the longest time lapse between the probes reaching target temperature was 24 minutes in trial 1 and 14 minutes in trial 2 (Table 2).

None of the treated cysts hatched when exposed to the hatching stimulant and no new cysts developed on the potato plants that were inoculated with non hatched eggs from treated cysts (Table 3). The number of juveniles hatching from the non-treated cysts in soil and without soil were 5,017 and 6,352 respectively. The number of new cysts that developed on plants inoculated with hatched juveniles and non-hatched eggs from the non-treated cysts were 776 and 761 respectively.

Steam treatment to free items of golden nematode infestation offers several important advantages over the traditional methyl bromide treatment. Most importantly, steam treatment is 100% environmentally safe and it has no ozone depletion potential. Further it is completely operator-safe requiring no safety equipment other than gloves. In contrast, methyl bromide treatment requires a self-contained breathing unit as well as protective clothing for operator safety. Steam treatment is significantly more time efficient requiring no more than two hours to complete while effective treatment with methyl bromide requires 24 hours. Steam treatment is significantly more cost effective and leaves no harmful or hazardous residues.

APHIS Methods Development specialist used these results in a proposal to add steam as an alternative treatment to methyl bromide for treatment of golden nematode infested equipment in the USDA, APHIS Plant Protection and Quarantine Golden Nematode Treatment Manual.

Table 1. Maximum temperature achieved and duration at 60ºC.

	Trial 1		Trial 2	
Location of probes	**Maximum temp. ºC**	**Duration ≥ 60ºC**	**Maximum temp. ºC**	**Duration ≥ 60ºC**
Inside battery box[1]	66.6[2]	1 hr	70.8	1 hr
Foot rest right side of tractor[1]	71.1	1 hr 16 mins	71.1	1 hr 6 mins
Axle – left side back[1]	70.0	1 hr 10 mins	71.1	1 hr 6 mins
Foot rest left side[1]	69.9	1 hr 10 mins	71.2	1 hr 6 mins
Right front axle[1]	69.3	1 hr 14 mins	71.1	1 hr 4 mins
Right side rear axle[1]	71.1	1 hr 12 mins	71.1	1 hr 2 mins
Sampling bag carrier	70.8	1 hr 18 mins	71.1	1 hr 2 mins
Bottom soil sampler wheel left[1]	71.2	1 hr 16 mins	72.7	1 hr 6 mins
Left tractor hitch point[1]	72.4	1 hr 24 mins	72.8	1 hr 14 mins
Tractor seat	71.9	1 hr 18 mins	72.8	1 hr 8 mins
Top of transmission[1]	70.3	1 hr 2 mins	70.3	1 hr 4 mins
Folded wind breaker	71.3	1 hr 14 mins	72.3	1 hr 6 mins
Middle soil sample hitch[1]	72.7	1 hr 24 mins	72.8	1 hr 16 mins
Behind tractor plate	71.8	1 hr 18 mins	72.7	1 hr 8 mins
Bottom of radiator	71.6	1 hr 14 mins	72.5	1 hr 6 mins

[1] Location of golden nematode cyst packets.
[2] Temperature probe was touching plastic battery case.

Table 2. Maximum temperature achieved inside tent and duration at 60ºC.

	Trial 1		Trial 2	
Location of probes	**Maximum temp. ºC**	**Duration ≥ 60ºC**	**Maximum temp. ºC**	**Duration ≥ 60ºC**
Left front bottom	68.2	1 hr	70.9	1 hr
Back of tent center	72.4	1 hr 22 mins	72.7	1 hr 12 mins
Back of tent bottom	72.5	1 hr 22 mins	72.8	1 hr 12 mins
Left side center	71.7	1 hr 20 mins	72.2	1 hr 10 mins
Right front bottom	70.7	1 hr 8 mins	71.8	1 hr 4 mins
Right back bottom	71.0	1 hr 18 mins	72.0	1 hr 6 mins
Right side center	72.3	1 hr 20 mins	72.8	1 hr 8 mins
Top front of tent	70.2	1 hr 10 mins	71.7	1 hr 6 mins

Table 3. Viability and infectivity of the golden nematode after exposure to steam at 60-71ºC for one hour.

Treatment of cysts	No. hatched juveniles	No. unhatched eggs that appeared viable	No. new cysts
Steam in soil	0	36	0
Steam no soil	0	5	0
No steam in soil	5,017	28	776
No steam no soil	6,352	30	761