REPLACING METHYL BROMIDE WITH A STEAM TREATMENT IN THE GOLDEN NEMATODE CONTROL PROGRAM

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Methyl bromide is used routinely as a regulatory treatment in the golden nematode control program to decontaminate items infested with the golden nematode when they are moved from regulated areas to nonregulated areas. Because of the effectiveness of methyl bromide, there has been essentially no effort to develop other types of treatments for this program. When the use of methyl bromide is discontinued, other types of treatments will be needed to ensure the integrity of the golden nematode quarantine.

The golden nematode is relatively insensitive to heat when in a desiccated condition but becomes highly sensitive when hydrated. We found that exposure of golden nematode cysts that had been presoaked in water to 55°C was lethal to the encysted eggs. In contrast, eggs in dry cysts tolerated temperatures as high as 75ºC for brief periods. Such studies suggested that heat had potential for decontaminating items infested with the golden nematode provided the cysts are in a moist condition.

Later studies on the effect of different sources of heat on golden nematode survival showed that solar heat under clear polyethylene for 8-16 hours is not lethal to the golden nematode consistently enough for a decontamination treatment in a quarantine setting. Dry heat under polyethylene did not consistently kill the nematode eggs even though temperatures achieved were supposedly lethal. Steam heat (60-65°C for one hour) under polyethylene was consistently lethal to encysted eggs and equaled methyl bromide in disinfesting equipment contaminated with the golden nematode. Because prewashing contaminated equipment with high pressure water did not cause increased kill of encysted eggs exposed to dry heat, it appeared that moisture to sensitize the eggs to high temperature was provided by steam. Later experiments demonstrated that exposure of encysted eggs to steam at 60°C from one to two hours killed 100% of the nematode eggs and that steam at 55°C for one hour completely inhibited hatching of encysted eggs. In initial attempts to establish time/temperature limits on the use of steam in the golden nematode program, treatments less than 55°C for one hour did not reduce the viability of golden nematode eggs sufficiently to meet the requirements of a regulatory treatment.

Experiments in 2000 focused on determining the time/temperature boundaries for a steam treatment that would meet the requirements of a regulatory treatment for decontaminating equipment infested with the golden nematode. The source of steam was a steambath generator model SM-12 manufactured by the Steamist Company, Rutherford, NJ. This generator was equipped with a Model 4004-71 Paragon Electric timer and a Johnson Control thermostat Model A319 with a range of 40-100ºC. To adequately distribute the steam, the generator was plumbed with one-inch steel piping that extended in a U-shape for 6 feet from the generator. The pipe was drilled with 1/16 inch holes at 8-inch intervals.

The equipment used in these experiments was a Farmall Cub tractor. Nylon sackettes containing five golden nematode cysts each in one gram of soil were placed in five locations on the tractor. The tractor was sealed inside polyethylene (6 mil). Steam was applied under the polyethylene to achieve temperatures of 55°C, 60°C and 65°C that were maintained for 45 minutes, one hour, and one hour and 15 minutes each. Temperature under the polyethylene was recorded with a thermocouple temperature recorder model KTX with a range of 0-100ºC that was manufactured by the Dickson Company. The experimental control consisted of cysts contained in soil-filled nylon sackettes that were not subjected to steam.

After the treatments were complete, cysts were retrieved and subjected to a hatching test. The hatching test consisted of soaking the cysts in water for five days then placing them in potato root diffusate (PRD) in ELISA plates for three weeks to stimulate hatch. The number of juveniles that emerge were counted weekly and fresh PRD was added. After three weeks of hatching, the cysts were crushed and the number of viable and non-viable eggs remaining determined. The eggs were then used to inoculate potato plants. After 12 weeks, the plants were examined for nematode reproduction.

When subjected to a hatching stimulant (PRD), no juveniles hatched from cysts that were exposed to steam treatments of 55°C, 60°C, or 65°C for 45 minutes, one hour, or one hour and 15 minutes each (Table 1). An average of 160 juveniles/cyst hatched from similar cysts that were not exposed to steam.

Most of the eggs in steam treated cysts that did not hatch appeared to be nonviable. When these eggs were placed on potato plants no cysts developed, indicating that steam treatment rendered the eggs nonviable. Several cysts developed on the potato plants that were inoculated with the remaining unhatched eggs in cysts that were not subjected to steam.

These data indicate that a steam treatment of 60°C for one hour is consistently effective enough to be used as a treatment in a quarantine setting for decontaminating items infested with the golden nematode. The results indicated that this treatment provides for 7°C and 15 minute buffers on either side of the desired time/temperature regime of 60°C for one hour and still remains effective. The treatment did not adversely affect the normal operation of the equipment treated. Field testing of this treatment in a regulatory setting will begin in the fall of 2000.

Table 1

Effects of Steam on Golden Nematode Survival

Temperature regime (°C)	Temperature range (°C)	No. of Hatched Juveniles Exposure Time (hr) 0.75	1.0	1.25	0 (control)
55	53.0 – 58.6	0	0	0	171
60	58.8 – 64.9	0	0	0	173
65	63.5 – 67.3	0	0	0	134