Inactivation of Verticillium dahliae in Peppermint Stems by Propane Gas Flaming

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ABSTRACT

In laboratory tests, internal stem temperatures of 60 C or higher killed 95 to 100% of the population of *Verticillium dahliae* in heavily infected stems of peppermint, *Mentha piperita*. In field tests using a boom-type field flamer at tractor speeds of 2.0 to 2.7 mph, internal stem temperatures usually reached or exceeded 60 C, and the average kill of the fungus was 99.5 and 99.2%, respectively. Speeds up to 2.7 mph also resulted in adequate incineration of infected plant debris on the ground, which could serve as an additional source of inoculum. Results of assays to determine the fate of the fungal propagules which persisted after flaming showed that few were viable after 1 week in the field; only three of 38 stems assayed over a 14-week period contained *Verticillium*, and only one showed an increase in propagule numbers. Flaming had little effect on soil temperature. At 2.0 mph, soil temperature increased 11.5 C at a depth of 1 mm, and only 1.0 C at a depth of 10 mm. At faster speeds, temperature changes were even less.

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In 1962, Powelson & Gross (4) found that propane flaming killed *Verticillium albo-atrum* in potato stems. Horner & Dooley (1) reported in 1965 that flaming peppermint (*Mentha piperita* L. 'Mitcham') stubble after harvest reduced greatly the amount of *Verticillium dahliae* Kleb. (= *V. albo-atrum* Reinke & Berth. var. *menthae* Nelson) inoculum in infected stems. Flaming also incinerated fallen leaves and other dried plant debris on the soil surface, thus eliminating them as sources of inoculum. Preliminary observations indicated that flaming greatly reduced build up and spread of Verticillium wilt in the field when it was used before the disease became widespread. Our objectives were to (i) determine internal stem temperatures required to kill *V. dahliae*; (ii) determine ground speeds in relation to gas pressure required to obtain the desired internal stem temperatures; (iii) discover the fate of the surviving fungal propagules after flaming; and (iv) determine whether temperature changes under the soil surface during propane flaming would be sufficient to affect survival of *V. dahliae* and peppermint rhizomes.

MATERIALS AND METHODS.—Stems of commercial peppermint heavily infected by *V. dahliae* (average = 655,800 propagules/g stem tissue) were stripped of leaves and used in all studies. Preliminary tests revealed that the fungal propagules were evenly distributed within the central portion of each stem. Therefore, two adjoining sections (0.5 g) were taken from each stem; one section was flamed, then both were assayed by fragmentation and dilution (2) to determine the effect of flaming on the propagule density (propagules/g tissue).

Internal stem temperatures during flaming were measured with a telethermometer (Yellow Springs Instrument Co., YSI Model 42SC) with appropriate probes (YSI Series 400). In laboratory tests, the probe was inserted into the pith of the section to be flamed, and the stem was passed over the flame of a bunsen burner until the desired internal stem temperature was recorded on the telethermometer. Five replicates of both stem sections were then assayed, and the percentage kill of *V. dahliae* was calculated by determination of the differences in propagule populations between the control and heated portions.

For field tests, a 0.5-g section was again used as an unflamed control, and a 15-cm portion of the stem was flamed with a commercial boom-type propane flamer typical of those available. The probe was placed in a shallow hole in an open field, an asbestos sheet placed over the hole, and the tip of the probe extended up (3 cm) through a 3-mm hole in the asbestos sheet. The probe was then inserted into the pith of the 15-cm stem piece, and the propane flamer was passed over the peppermint stems at speeds varying from 1.0 to 4.8 mph using a gas pressure of 35 psi. The lead wire from the probe was buried

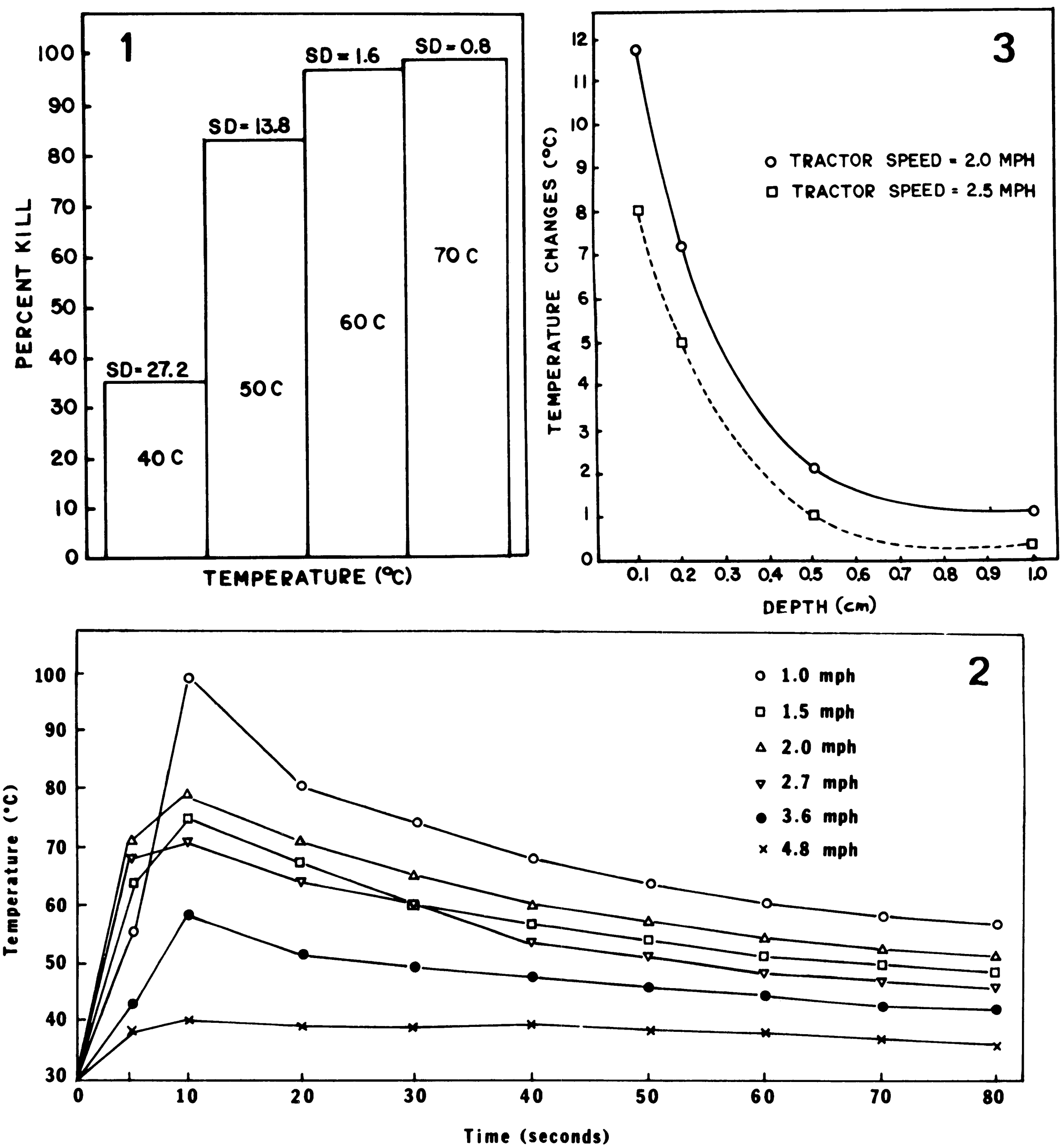

Fig. 1-3. 1) Average percent kill of *Verticillium* in infected peppermint stems at four temperatures (average number of fungal propagules/g stem tissue = 410,200). **2)** Typical internal temperature changes during an 80-sec time period (10 stems/treatment). **3)** Average temperature changes at various soil depths.

about 6 cm under the soil, and the temperature changes were observed on the telethermometer at specific time intervals after passage of the flamer. The portion of the stem seated in the asbestos sheet was discarded, then a 0.5-g section was removed from the base of the flamed stem and assayed along with the control portion of the stem. The percentage kill of the fungus was then determined.

To discover the fate of the surviving (residual) fungal propagules in peppermint stems after flaming, a field survival test was made. A 0.5-g section was removed from each flamed stem and assayed along with the control section, and the percentage kill of the fungus was calculated. The remainder of the flamed stems were placed on the surface of a peppermint field. Five stems were then collected on eight different occasions and assayed to determine the amount of *V. dahliae* remaining.

Temperature changes under the soil surface induced by the flamer were measured by a probe with a flat stainless steel disc. The probe was placed 1 to 10 mm under the soil surface, and temperature changes were observed during and after passage of the flamer at speeds of 2.0 and 2.5 mph, and a gas pressure of 35 psi.

RESULTS.—When stems were heated in a bunsen burner flame, an internal stem temperature of 60 C gave 97.6% kill of the fungal propagules (Fig. 1). Temperatures of 40 and 50 C gave average kills of 35.1 and 83.1%, respectively. These results indicated that an internal temperature of 60 C would be desirable when experiments were conducted in the field.

Field experiments were done on 26 September 1969, and 24 June and 18 August 1970. The flamer was operated at speeds of 1.0, 1.5, 2.0, 2.7, 3.6, and 4.8 mph, with a gas pressure of 35 psi. Internal stem temperatures were recorded before and at either 5- or 10-sec intervals after the flamer passed over the stems. Typical internal stem temperatures obtained at various flamer speeds during an 80-sec time period are shown in Fig. 2. Table 1 shows the average of the percentage kill of the fungus, and peak internal stem temperatures for the three different flaming dates. In all but one individual observation, at least 95% kill was obtained at speeds from 1.0 to 2.7 mph. At speeds greater than 2.7 mph, there was a large variation in the percentage kill.

Simple correlation coefficients showed that speed was significantly correlated (P = 1%) to both peak temperature and percentage kill, as was peak temperature with percentage kill. A multiple regression analysis showed that speed was having a greater effect upon percentage kill of the fungus than was peak temperature. Therefore, the entire temperature curve over time was constructed, and simple correlation coefficients between time above 45, 50, 55, 60, 65, and 70 C and percentage kill were determined. No significant correlation between temperature level and percentage kill was found.

The multiple regression analysis also showed that date of flaming was an important factor in achieving a high-percentage kill of the fungus. Temperature and wind conditions varied on the flaming dates, and probably influenced the effectiveness of flaming.

Stems initially infected with *V. dahliae* were flamed, placed in the field, and assayed at various dates after flaming. Only three of 38 stems contained *Verticillium* (Table 2). Of these three, only one showed an increase in the number of *Verticillium* propagules from the time it was placed in the field, recollected, and assayed.

Temperature changes under the soil surface after propane flaming were small (Fig. 3). At 2.0 mph, the temperature increased 11.8 C at a depth of 1 mm, and only 1.0 C at a depth of 10 mm. At 2.5 mph, the

TABLE 1. Peak temperatures and percent kill of *Verticillium dahliae* in infected peppermint stems on three separate dates at six different tractor speeds (5 stems/treatment; gas pressure = 35 psi)

Speed	Date	Avg no. of propagules/g tissue before flaming	Avg peak temperature	Avg kill
mph			*C*	*%*
1.0	26 Sept. 1969	440,200	100.0	98.3
	24 July 1970	370,060	70.4	99.8
	18 Aug. 1970	1,056,000	77.4	99.2
1.5	26 Sept. 1969	457,760	73.7	98.9
	24 July 1970	668,400	55.4	99.3
	18 Aug. 1970	1,120,000	79.0	99.8
2.0	26 Sept. 1969	466,160	79.8	98.7
	24 July 1970	544,120	55.8	99.7
	18 Aug. 1970	1,250,000	69.4	100.0
2.7	26 Sept. 1969	444,400	71.2	99.5
	24 July 1970	436,000	55.1	99.6
	18 Aug. 1970	1,120,000	69.8	98.6
3.6	26 Sept. 1969	431,200	59.3	98.3
	24 July 1970	250,000	47.5	67.1
	18 Aug. 1970	960,000	57.0	99.0
4.8	24 July 1970	482,100	41.0	18.0

TABLE 2. Survival of residual propagules of *Verticillium dahliae* in peppermint stems placed in the field after flaming (tractor speed = 2.0 mph; gas pressure = 35 psi; average number of propagules/g stem tissue before flaming = 420,000)

Days after flaming	Avg % kill after flaming	No stems with no *V. dahliae*	No. stem containing *V. dahliae*	% Increase (+) or decrease (–) in *V. dahliae*
7	98.8	4	1	–90.7
14	75.5	5	0	–
21	89.9	4	0	–
28	67.0	4	1	–98.5
35	54.4	4	1	+436.7
49	76.3	5	0	–
63	87.6	5	0	–
94	73.9	4	0	–

temperature changes at these depths were even less.

DISCUSSION.–From limited published data (1, 4), it was hypothesized that about 95% kill of *V. dahliae* inside infected stems would be required to control the disease in a field situation. Preliminary laboratory experiments indicated that an internal stem temperature of 50 C, which gave an average kill of 83.0% and a range from 58.3 to 99.4% was too low. The upper limit of this range substantially reduced the fungal propagule density, whereas the lower limit was too low. Internal stem temperatures of 60 C or higher decreased the density of fungal propagules drastically, and consistently killed 95% or more of the fungus (Fig. 1).

In field experiments, flamer speeds of 1.0 to 2.7 mph consistently killed more than 95% of the propagules (Table 1), and internal stem temperatures were 55 C or higher. Speeds up to 2.7 mph also incinerated the dried leaf and stem debris which were on the ground and could serve as a source of inoculum. Therefore, the total result of flaming was to eliminate the wilt fungus in dead plant debris by incineration, and to drastically reduce the density of fungal propagules in the erect stubble.

Internal stem temperatures did not correlate so highly with percentage kill as did speed, because most of the variation was associated with internal stem temperatures. The diameter and maturity of the peppermint stems would affect the internal stem temperatures. The heat from the propane flamer is highly variable (3), and therefore the positioning of the probe in the stem would also introduce variation. If the probe happens to be at a "cooler" or "hotter" spot during different runs, the observed temperature could be misleading. These factors, and possibly others, could explain why speed, which is regulated, is more closely correlated with percentage kill than is temperature.

The results of the residual assay, showing that nearly all of the residual propagules die within 1 week after flaming, were surprising. The fungal propagules present immediately after flaming may have been injured and, therefore, slowly died from heat shock. Another possible explanation is that *V. dahliae*, which is in a vegetative, parasitic stage at the time of flaming, cannot adjust rapidly enough to form its saprophytic or dormant stage in the host tissue killed by flaming and, therefore, perishes.

Weather conditions after the flaming process may also affect the residual propagule density. The weather was warm (30 C) and dry for several weeks after the stems in this experiment were placed in the field. Rapid drying of the stem tissue could have been responsible for rapid disappearance of the residual propagules. The small number of stems studied severely hampered interpretation of this data. Additional data are needed before the true fate of the surviving fungal propagules after flaming can be determined accurately.

Peppermint produces both stolons and rhizomes. Because most of the aboveground stolons do not survive normal winter conditions, the effect of flaming on them is of little or no significance. The underground rhizomes are important for regrowth of the peppermint crop the following spring. The data (Fig. 3) show that at 2.0 mph there was a 1.0-C change at a depth of 10 mm, and at 2.5 mph there was a 0.3-C change at the same depth. These temperature changes are of little or no consequence to the rhizomes at this depth, and many rhizomes at lesser depths should also survive. Therefore, flaming at 2.0 or 2.5 mph is of little or no consequence to the peppermint plants, but it does kill about 95% of the propagules of *V. dahliae*, thus drastically reducing the amount of fungal inoculum.

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