AN ABSTRACT OF THE THESIS OF

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Title: INACTIVATION OF *VERTICILLIUM DAHLIAE* IN PEPPERMINT STEMS BY PROPANE GAS FLAMING

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The fungus *Verticillium dahliae* Kleb. causes a wilt disease of peppermint, *Mentha pipertita* L. Propane flaming of peppermint stubble is widely used to control *Verticillium* wilt in the Willamette Valley of Oregon, but little is known about the temperature required for effective control of the fungus.

The purposes of this study were: (1) to determine the internal stem temperatures required to greatly reduce the population of *V. dahliae*; (2) to determine the tractor speeds and propane gas pressure required to obtain these internal stem temperatures; (3) to discover what happens to the surviving fungal population after flaming; and (4) to measure temperatures under the soil surface during propane flaming and determine whether these might affect survival of the peppermint rhizomes.

In preliminary laboratory experiments, exposure of stems to a flame until the internal temperature reached 50C killed an average of

83.1% of the Verticillium propagules; percent killed ranged from 58.3 to 99.9%. Though the upper limit of this range was considered adequate to control Verticillium, the average kill and lower limit of the range were considered inadequate. Average mortality at 60C was 97.6% with a range from 94.3 to 100%. This and higher temperatures should be adequate to control the disease.

Field experiments showed that tractor speeds of 2.0 and 2.5 mph at 35 psi gas pressure resulted in an average of 99.5 and 99.2% kill of the fungus, respectively, and adequately eliminated infected plant debris on the ground. The slower speed should be used on cool mornings or days, and the higher speed should be used on warmer days.

A multiple regression analysis of data obtained from field tests showed that speed was the best indicator of percentage kill of the fungus. Peak temperature was correlated with both speed and percentage kill of the fungus at the 1% level.

The results of assays to determine the fate of the surviving population of Verticillium immediately after flaming showed that little or none of this population remained after one week in the field. Flamed stems were placed in the field and periodically assayed over a 14 week period. Three of 38 stems contained Verticillium, and only one showed an increase. In every case where the fungus survived flaming, a high population (80,000 propagules per gram tissue or more) of Verticillium was present immediately after flaming.

Soil temperature recordings showed that the temperature decreased with depth. At 2.0 mph, there was an 11.8C increase at a depth of 0.1 cm and only a 0.1C change at a depth of 1.0 cm. At 2.5 mph, the temperature changes at these depths were even less. At depths around 1.0 cm, where rhizomes important for the regrowth of the next year's crop exist, the temperature changes were not great enough to injure them.

Inactivation of *Verticillium dahliae* in Peppermint Stems by Propane Gas Flaming

by

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDIX TABLES

INACTIVATION OF VERTICILLIUM DAHLIAE IN PAPPERMINT STEMS BY PROPANE GAS FLAMING

INTRODUCTION

Verticillium dahliae Kleb. (= V. albo-atrum var. menthae Nelson) causes a wilt disease in peppermint (Mentha piperita L. cv. Mitcham). It virtually eliminated peppermint production in the Mid-west, and thereby caused peppermint to become a major agricultural crop in the Pacific Northwest. Peppermint wilt first became important in the Pacific Northwest in 1951, and has since spread to many thousands of acres (Horner and Dooley, 1965). Various control methods have been tried, but none has proven entirely satisfactory. Soil fumigation (Horner and Dooley, 1966) is effective but economically infeasible for large acreages. Production of wilt resistant peppermint by irradiation (Sherrod, 1969) is a potentially useful innovation, but resistant strains are still to be released commercially. Because the microsclerotia of V. dahliae may lie dormant in the field for many years (Nelson, 1950), certification of disease-free planting stock which is now being performed, is not feasible as a control measure over a long period of time. Thus, propane flaming (Horner and Dooley, 1965), which is widely used in the Willamette Valley of Oregon, remains the most valuable control method.

Powelson and Gross (1962) reported that propane flaming killed Verticillium in potato stems. Horner and Dooley (1965) found that flaming mint stubble after harvest reduced greatly the amount of V. dahliae inoculum in infected stems. Flaming killed the fungus within stems without incinerating them. 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 buildup and spread of Verticillium wilt in fields where it was applied before the disease became widespread. Other than this report, and observations over the past five years that propane flaming does control Verticillium in a field, no further experimentation has been done on this procedure.

The purposes of my study were:

1. To find what internal peppermint stem temperatures are needed to kill Verticillium dahliae.

2. To find at what speeds propane flaming will cause the desired internal stem temperatures.

3. To discover what becomes of the surviving fungal population after flaming.

4. To find what temperature changes occur under the soil surface during propane flaming and whether these might effect survival of the peppermint rhizomes.

LITERATURE REVIEW

Though the term "Thermal Agriculture" has been coined only recently (Swanson, 1968), the use of heat for killing weeds and insects has been practiced for over a century. The first flame cultivator was designed and patented by John A. Craig of Columbia, Arkansas (Edwards, 1964). Edwards (1964) stated that this development led to numerous patents on similar devices, and in 1900 a patent was granted on the first machine claimed to be an "insect killer." Many improvements were made on the design of this machine, and in 1935 the first successful attempt in flame cultivation was achieved. From its original use as a "weed killer" and later as an "insect killer," propane flaming is now used for such diversified agricultural problems as: (1) alfalfa weevil control (Bennett and Luttrel, 1966; Burbutis, 1966; Clark and Simpson, 1966; Swanson, 1968); (2) weed control (Clark and Simpson, 1966); (3) control of diseases in peppermint (Coykendall, 1968; Horner, 1965; Horner and Dooley, 1965, 1966); (4) potatoes (Powelson and Gross, 1962); (5) hops (Talboys, 1961); (6) beets (Maloy, 1970), and (7) alfalfa (Maloy, 1970); (8) control of pathogens associated with grass seed production (Hardison, 1960); (9) control of dodder (Torell, 1968); (1) defoliation of corn (Batchelder and Porterfield, 1969; Porterfield and Batchelder, 1970); and (11) delaying the flowering of inbred lines of legumes (Trybom and Vanderlip, 1970).

In hops, potatoes and peppermint, control of Verticillium wilt by heat has been used (Horner and Dooley, 1965; Luck, 1953; Powelson and Gross, 1962; Talboys, 1961). Studies were made by Luck (1953) on the thermal deathpoint of the spores and microsclerotia on the peppermint strain of Verticillium. The experiments were performed at constant temperatures in a hot water bath, and it was found that there was a significant difference between the resistance of spores and microsclerotia to moist heat. At 49C, no viable spores remained after 20 minutes, but at the same temperature it took 40 minutes to eliminate all of the microsclerotia.

If Verticillium infected hop vines are returned to a field after harvest, there is a chance that an epidemic will occur (Talboys, 1961). Talboys (1961) found that if the vines were composted at various temperatures before being returned to the field, the fungus could be eliminated. At 60C the fungus was killed in 15 minutes, and at 40C it took seven days to eliminate the fungus.

The first report of thermal inactivation of Verticillium by propane flaming was on V. albo-atrum in potatoes (Powelson and Gross, 1962). The internal stem temperatures were measured by inserting short melting point tubes filled with powdered "Temp-pils" (mp 125F, 150F, and 200F) into the pith of diseased potato stems. At a tractor speed of 3 mph, when 60% of the 125F tubes were melted, 20% of the 150F tubes were melted, and none of the 200F tubes, Verticillium was killed in 50% of the stems. At a speed of 1.5 mph, when the percentage of 125F, 150F, and 200F tubes melted was 100, 90 and 53% respectively, Verticillium was killed in 95% of the stems. It appeared from this data that propane flaming could reduce the Verticillium population in potato stems.

In 1965, peppermint stubble was flamed with a propane field burner to determine if flaming would kill Verticillium dahliae Kleb. in infected stems (Horner and Dooley, 1965). Flaming at 1 mph and 35 psi gas pressure nearly eliminated the fungus from infected stems. Flamer speed of 2 mph at 35 psi gave 80 and 89% kill in wet and dry stems, respectively. Speeds above 2 mph were much less effective. This data indicated that propane flaming could be used as an effective control of Verticillium in peppermint.

The use of propane gas is a desirable method of disease control because it is relatively efficient and inexpensive. The liquid propane gas which is used in flame cultivation creates combustion at the point of contact; combustion does not occur within the torch itself (Swanson, 1968). The advantages of propane flaming listed by Swanson (1968) are:

a) there is no residue left on the crop or soil;

b) adjoining crops are not affected;

c) it does not require incorporation into moist soil for herbicidal action;

d) it is not affected by soil types or management;

e) it is not subjected to light activity;

f) it produces an immediate effect;

g) it can be repeated as often as necessary.

In extensive studies on flame cultivation (Perumpral, Lien and Liljedahl, 1966) some of the important factors affecting results were as follows: fuel pressure, surface conditions, burner adjustment, type of fuel, speed of application, and atmospheric conditions. It was originally believed that if a hotter flame was needed, the fuel pressure should be increased (Thomas, 1964). Perumpral, Lien and Liljedahl (1966) found that an increase in fuel pressure to a certain level will increase both the length and width of the flame, but any increase in pressure above the optimum is no longer significant. Thomas (1964) noted that if the flaming is done on a rough and bumpy surface, the flame is uneven and crop injury may occur. Perumpral, Lien and Liljedahl (1966) found that the burner angle required is different for all crops and all conditions, with the best burner angle being 30-45^{o}. Beyond 45^{o} turbulence occurs and an uneven burn results. Thomas (1964) states that the type of fuel used is important because vapor pressure is a limiting factor. Most often either propane, butane, or a mixture of the two is utilized. Swanson (1968) mentions that the speed of travel must be adjusted to the heat intensity of the flame and the tolerance of the row crop. The speed must also be adjusted to meet the prevailing atmospheric conditions. In cool air or high humidity, a higher gas pressure (to the optimum), or a slower speed should be used to increase the temperature around the plants. Hovers may also be used to contain the heat around the plants for a longer period of time (Bennett and Luttrell, 1966).

Glenn Page, Agricultural Engineer at Oregon State University, noted a large amount of variability in the flame emitted by the propane flamer. At ground level the flame induces only a slight change in the ambient temperature. Because the energy from the flame causes the soil moisture to form steam, this immediately limits the maximum soil surface temperature to 100C. The hottest point of the flame appears to be about 1" above ground level (Thomas, 1967) and then the temperature declines the further above the soil it is measured.

Because Verticillium exists internally in peppermint stems, it is naturally important to increase the internal stem temperature to a point where most or all of the fungus is eliminated. Thomas (1967) noted that a relatively long exposure to a relatively high temperature is required to bring about an appreciable temperature rise at a relatively shallow depth in a plant stem. A 1/4 second exposure of a corn plant to flames from four standard burners (agricultural types) produced a maximum temperature of approximately 115F at a depth of 1/16" from the surface. It was suspected that the moisture vaporization formed a very thin protective or cooling film which prevented a rise of temperature above approximately 200F at the surface of a living, succulent plant. Only after this free water evaporated could the external stem temperature rise above this point, thereby raising the internal stem temperature.

What occurs to the plant from the flaming process is also of importance. With peppermint, because the flaming occurs after harvest, it is preferable to kill the above ground portions of the plant, thereby eliminating a possible place for the residual fungal population to increase. By killing these tissues, Verticillium may also die even though not directly killed by heat because it may be unable to change quickly enough from its parasitic to saprophytic stages. It has been stated by Swanson (1968) and others that propane flaming disrupted the plant cells by causing the liquid in them to boil. It was later hypothesized by Thomas et al., 1968, and others) that the death of a functioning cell results at a temperature at which there is coagulation of the cytoplasm. This theory has also been disputed (Daniell, Chappell and Couch, 1969) because in some plants death begins at 40C, but much higher temperatures are needed for coagulation to occur. It was further noted that there appear to be proteins which are relatively stable at high temperatures. Daniell, Chappel and Couch (1969) further proposed the lipid liberation theory. With this theory, high temperature injury, whether reversible or irreversible, is attributed to the melting of lipid constituents in the cell or cell membrane. This theory is linked with the observation that lipid formed by living organisms at high temperatures is more solid than lipid formed at lower temperatures. Changes of lipids within the cell membranes could account for the membrane changes at high temperatures.

Although the reasons for the killing of plant tissues are still being disputed, Thomas *et al*. (1968) states that there is injury to the conductive tissues and cambial layers in the plant, and this ultimately causes the death of the above-ground portions of the plant. The death of these tissues may ultimately determine if the residual *Verticillium* population will be able to survive or if it too will die.

MATERIALS AND METHODS

Plants

Stems of commercial peppermint infected by *Verticillium dahliae* were used in all studies. They were obtained from the commercial fields of Wayne Chambers, five miles northwest of Albany, Oregon. Plants were selected that had visible symptoms of infection. Though the symptoms of *Verticillium* infection are highly variable in peppermint, if several of the following disorders are evident, one can be certain that a high population of *Verticillium* will be present in the stems. These symptoms (Nelson, 1950, p. 40-54) are:

A. Retarded elongation

B. Loss of leaf lustre

C. Shoot and leaf assymmetry

D. Twisting and curling of leaves

E. Blanching - all green in the leaf disappears

F. Chromatism - upper whorls of leaves may be colored

G. Wilting and defoliation of plant.

Assay Procedures

The peppermint stems were stripped of all leaves and a 0.5 g sample was then cut from the basal portion of each. This sample served as an unflamed control for that stem. The propane flaming treatment was performed on the remainder of the stem, then another

0.5 g section was cut from the base of the flamed stem. In this manner, two adjoining sections of the original stem were assayed, one which had been flamed and one which had not. The number of *Verticillium* propagules [1] in both sections could then be determined, and the percent kill caused by the treatment could be calculated.

The assay consisted of cutting each 0.5 g stem section into 1.0 mm pieces, then grinding these with 20 ml of sterile distilled water for 60 seconds at 100 volts in an Omni-mixer. Aliquots, representing specific dilutions of the 0.5 g stem section, were then pipetted into 50 ml of cooled (42 C) ethanol streptomycin agar medium (Nadakavukaren and Horner, 1959). The dilutions were commonly 1:20 and 1:400. The agar was then poured into five separate petri plates, and the plates were stored at room temperature in the dark for a minimum of ten days. The plates were then removed and the colonies of *Verticillium* were counted (Nadakavukaren and Horner, 1959). The number of propagules per gram of stem tissue could then be calculated.

Propane Flamer

A commercial propane flamer, typical of those available to mint growers in Oregon, was used (Figure 1). The flamer consists of a boom about 5.5 m long with 45 gas nozzles divided equally into three

[1] In this thesis, the word propagule means any portion of the fungal thallus which will grow into a new plant body when placed on the assay medium used.

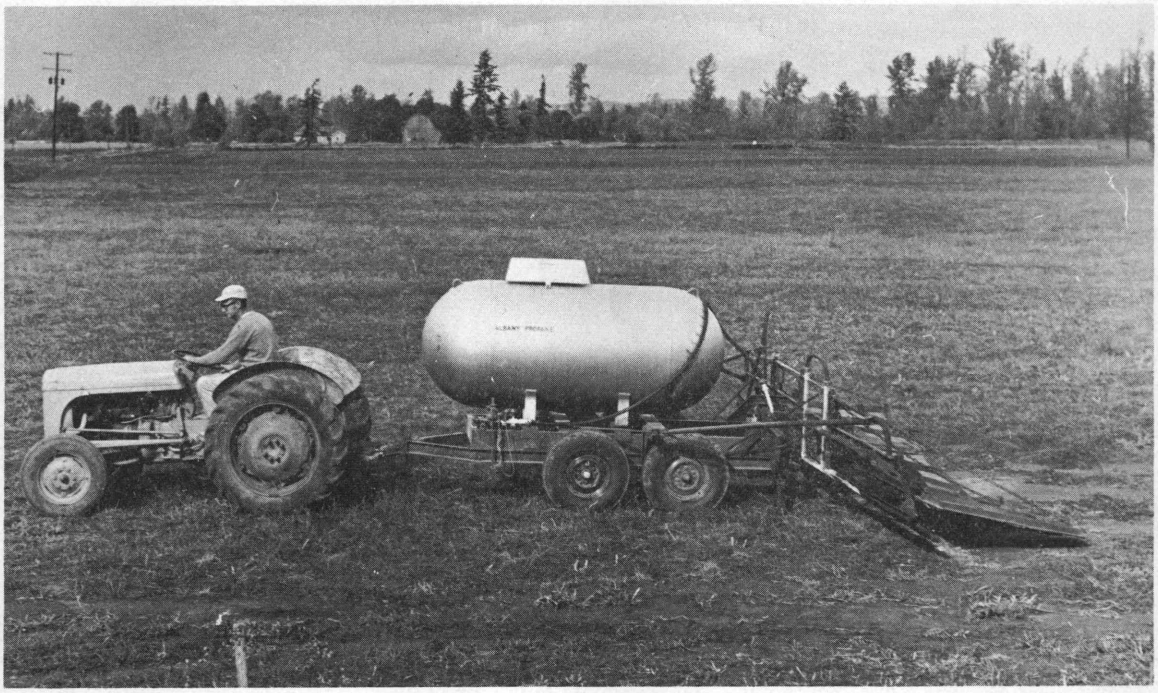

Figure 1. Propane flamer similar to the one used in these experiments.

sections. The nozzles have an internal diameter of 4.0 cm and are spaced at 11.4 cm intervals. The three sections of nozzles are each covered by a 1.4 x 1.8 m metal plate (hover) designed to keep the flame close to the ground and help contain the heat around the peppermint stems. The hovers are held about 18.0 cm off the ground by metal skids and the gas boom is hinged to allow the flame emitted to strike the ground from a fairly constant height and angle. Only one flamer was utilized because of the large number of other variables which were inherent in the design of the experiments.

Recording Internal Stem Temperatures

A "telethermometer" (Yellow Springs Instrument Model 42SC) with appropriate probes was used to measure internal stem temperatures. This instrument has a low range of -40 to +30C, a middle range of +20 to +80C, and a high range of +70 to +150C. According to the manufacturer, this instrument is accurate to ± 0.5C at -20 to +120C. The readability in this range is 0.2C, and the reproducibility is ± 0.5C.

YSI series 400 probes with 3.1 m leads were used with the recorder. The stainless steel, solder sealed probes were 7.6 cm long, with the thermocouple located at the tip of the probe. According to the manufacturer, the time constant of the probes is 0.6 seconds, and their temperature range is -40 to +150C.

Preliminary Laboratory Experiments

Before experiments were performed in the field, tests were conducted in the laboratory to determine internal stem temperatures required for adequate kill of Verticillium. The YSI probe was inserted into the pith of a 0.5 g stem section, and the stem was heated in the flame of a bunsen burner until the desired internal temperature of 40, 50, 60, or 70C registered on the telethermometer. The stems were then assayed as described earlier.

In calculating percentage kill of Verticillium in each peppermint stem, it was assumed that the fungus was evenly distributed between two adjacent 0.5 g stem sections. To test this assumption, stems were gathered, and the two adjacent 0.5 g sections from each were assayed to determine if the fungus was in fact evenly dispersed. If the population of Verticillium was about equally distributed in each section, the assumption was considered valid.

Field Experiments

Field experiments were performed in the following manner (Figure 2). A 0.01 x 0.31 x 0.01 m asbestos board with a 3.0 mm hole in the center was placed over a shallow hole in the ground. A probe was placed so that about 3.0 cm of it extended up and through the aperture in the asbestos board. The probe was then inserted about 3.0 cm into the pith of a stem 15.0 cm in length. The stem was

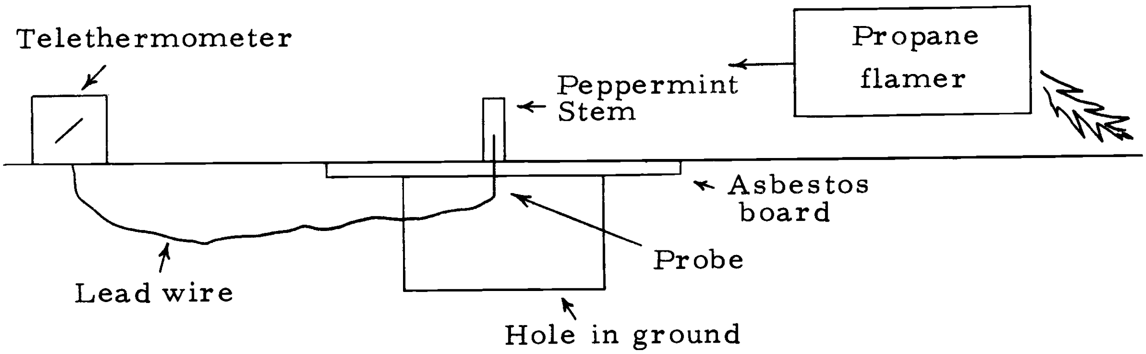

Figure 2. Arrangement of peppermint stem, probe, and telethermometer during field experiments.

then forced into the opening in the asbestos board to prevent any portion of the probe from being exposed directly to the propane flame, and the lead wire from the probe to the telethermometer was buried under about 2.5 cm of soil. The propane flamer was then driven over this arrangement, and any temperature changes within the stem were noted on the telethermometer at specific time intervals. The propane flamer was pulled by a tractor at speeds varying from 1.0 to 4.8 mph. The gas pressure was kept constant at 35 psi.

Residual Assay

Flamed stem sections usually carry a residual population[2] of *Verticillium* (Horner and Dooley, 1965). Because *V. dahliae* can grow as a saprophyte on dead mint stem tissue, it was deemed important to determine what happened to the residual population in flamed stems. Eighty infected stems were cut into two sections. One section was assayed and the other section was flamed at a speed (2.0 mph) calculated to give a high kill (95-100%) of the *Verticillium* propagules. A portion of each flamed stem was assayed to determine the initial percentage kill of the fungus. The remaining portion of each stem was then placed in an experimental plot of peppermint at the Oregon State University Botany and Plant Pathology Farm on July

[2] Residual population is used to denote that portion of the *Verticillium* population present immediately after flaming.

1, 1970. The stems were placed under a wire screen to protect them from being inadvertently moved. Five stems were collected and assayed on the following dates:

Date	No. of days in field
7-28-70	7
8- 4-70	14
8-11-70	21
8-18-70	28
8-25-70	35
9- 8-70	49
9-22-70	63
10-24-70	99

The final assay was modified to make it more sensitive in detecting *Verticillium* among the large population of saprophytes that had developed. The entire stem pieces were ground with water as usual, then dispersed into 500 ml assay medium from which 37 plates were poured.

Temperature Changes Under the Soil Surface

To determine the temperature changes under the soil surface as the propane flamer passed over, a probe with a flat stainless steel disc was utilized. This probe has a time constant of 1.7 seconds and a temperature range of -40 to +100C, according to the manufacturer. The probe was placed at depths of 1.0 to 10.0 mm under the soil surface, and the temperature changes at these depths were recorded as the propane flamer passed over at various speeds.

Statistical Analysis of Data

Because of the large amount of data collected, computer analysis of the data was used. The most useful computations were simple correlation coefficients and F values obtained from a multiple regression analysis. For a multiple regression analysis, the computer finds the independent variables which best fit the equation $\hat{Y} = a + b_1x_1 + b_2x_2 + . . . + b_nx_n$. From this equation predictions may be made on future experiments (Steel and Torrie, 1960). As each independent variable enters the equation, an F value is given by the computer. This F value shows if the independent variable which is entering the equation has any significance in predicting the value of the dependent variable. In this manner, for example, it can be determined if the speed of the propane flamer or the internal stem temperature has the most affect on reducing the *Verticillium* propagules.

RESULTS

Preliminary Laboratory Experiments

Kill of V. dahliae propagules obtained by heating stems in a bunsen burner to various internal temperatures is shown in Table 1 and expressed graphically in Figure 3.

When the stems were heated to 40C, the average kill was 35.1% with a range from 8.2 to 60.3%. At 50C the average kill was 83.1% with a range from 58.3 to 99.9%; at 60C the average was 97.6% kill with a range from 94.3 to 100%; at 70C the average was 99.3% kill with a range from 97.4 to 100%.

Percentage of the total Verticillium population in adjacent 0.5 g peppermint stem sections is shown in Table 2. The average for the five stems was 48.7% in the lower and 51.2% in the upper section. Therefore, the assumption that propagules of V. dahliae are about equally divided between the two sections seems valid.

Field Experiments

The field experiments were performed on three different dates:

1. September 26, 1969
2. July 24, 1970
3. August 18, 1970

The propane flamer was operated at speeds of 1.0, 1.5, 2.0, 2.7, 3.6,

Table 1. Percent kill of Verticillium in stems heated to 40, 50, 60, and 70C.

Stem	Temperature			
	40C	50C	60C	70C
	% kill	% kill	% kill	% kill
1	12.1	99.9	94.3	99.8
2	27.1	85.0	96.0	98.4
3	46.3	67.6	96.7	100
4	47.5	79.9	100	100
5	60.3	90.8	98.3	99.4
6	8.2	97.3	97.5	98.6
7	42.8	58.3	97.6	97.4
8	45.4	98.3	98.9	99.4
9	39.3	75.6	99.1	100
10	21.8	77.8	97.3	99.5
Average	35.1	83.1	97.6	99.3
Standard Deviation	27.2	13.8	1.6	0.8

Table 2. Distribution of Verticillium in adjoining peppermint stem sections.

Stem no.	% In lower 0.5 g section	% In upper 0.5 g section
1	45.9	54.1
2	41.6	58.4
3	51.4	48.6
4	51.9	48.1
5	53.0	47.0
Average	48.7	51.3

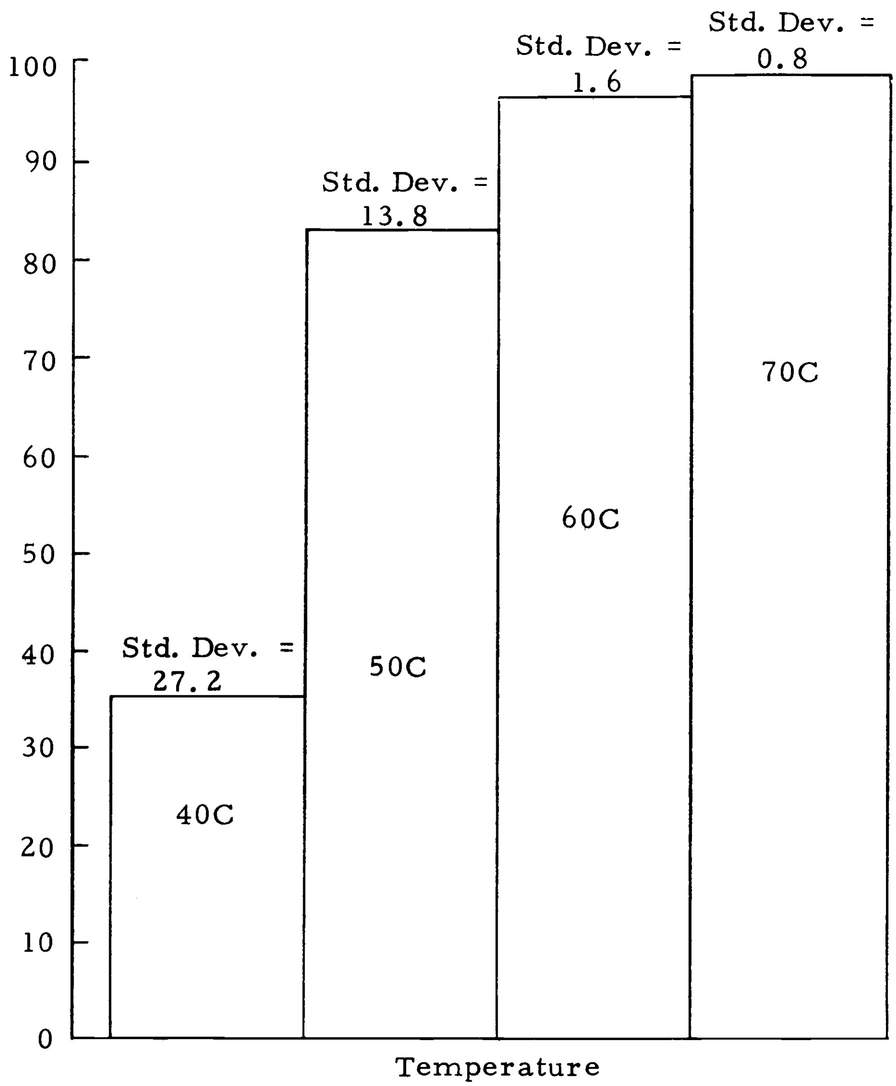

Figure 3. Average percent kill of Verticillium in peppermint stems at four temperatures.

and 4.8 mph. Internal stem temperatures were recorded at the beginning of each flaming and then at either five or ten second intervals after the flamer had passed over the stem. Flamed stems and their unflamed counterparts were then assayed to determine the reduction in Verticillium caused by the flaming. From these data, percentage kill of the fungus at various speeds and temperatures were calculated for all 77 observations (Appendix Table 1). Table 3 shows the averages of these values. The relationship of speed to percentage kill on the three different dates is shown graphically in Figure 4, and Figure 5 shows the relationship of speed to internal stem temperature on the three different dates.

In all but one case, there was at least 95% kill of Verticillium at speeds from 1.0 to 2.7 mph. At speeds higher than 2.7 mph there was a variation from 17.0 to 100% kill of the fungus. Figures 4 and 5 show that the internal stem temperature seems to be more closely related to the speed of the propane flamer than to percentage kill.

Results of the field experiments were shown to the Statistics Department at Oregon State University. They decided that a computer analysis would be beneficial. Simple correlation coefficients were obtained (Table 4). The variable speed^2 correlated better with the data than speed, but all simple correlation coefficients for speed^2 and speed are significant for the 77 observations. There was a lesser correlation between peak temperature and both final count and percentage kill.

Table 3. Peak temperatures and percent kill of *Verticillium* on three separate dates and six different tractor speeds (gas pressure = 35 psi).

Speed (mph)	Date	Average peak temperature (°C)	Average percent kill
1.0	9-26-69	100	98.3
	7-24-70	70.4	99.8
	8-18-70	77.4	99.2
1.5	9-26-69	73.7	98.9
	7-24-70	55.4	99.3
	8-18-70	79.0	99.8
2.0	9-26-69	79.8	98.7
	7-24-70	55.8	99.7
	8-18-70	69.4	100
2.7	9-26-69	71.2	99.5
	7-24-70	55.1	99.6
	8-18-70	69.8	98.6
3.0	9-26-69	59.3	98.3
	7-24-70	47.5	67.1
	8-18-70	57.0	99.0
4.8	7-24-70	41.0	18.0

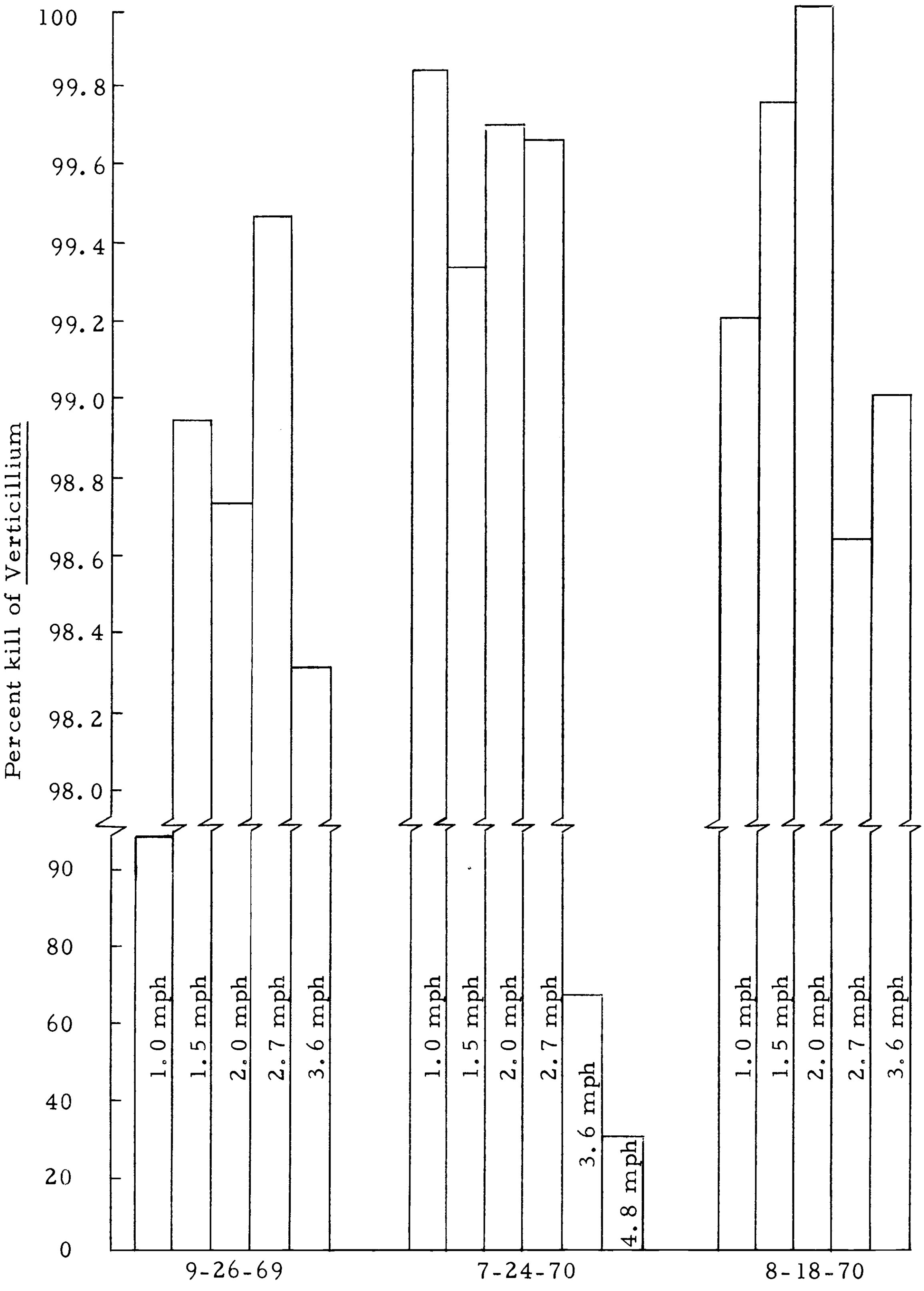

Figure 4. Speed versus average percent kill of Verticillium on three trial dates.

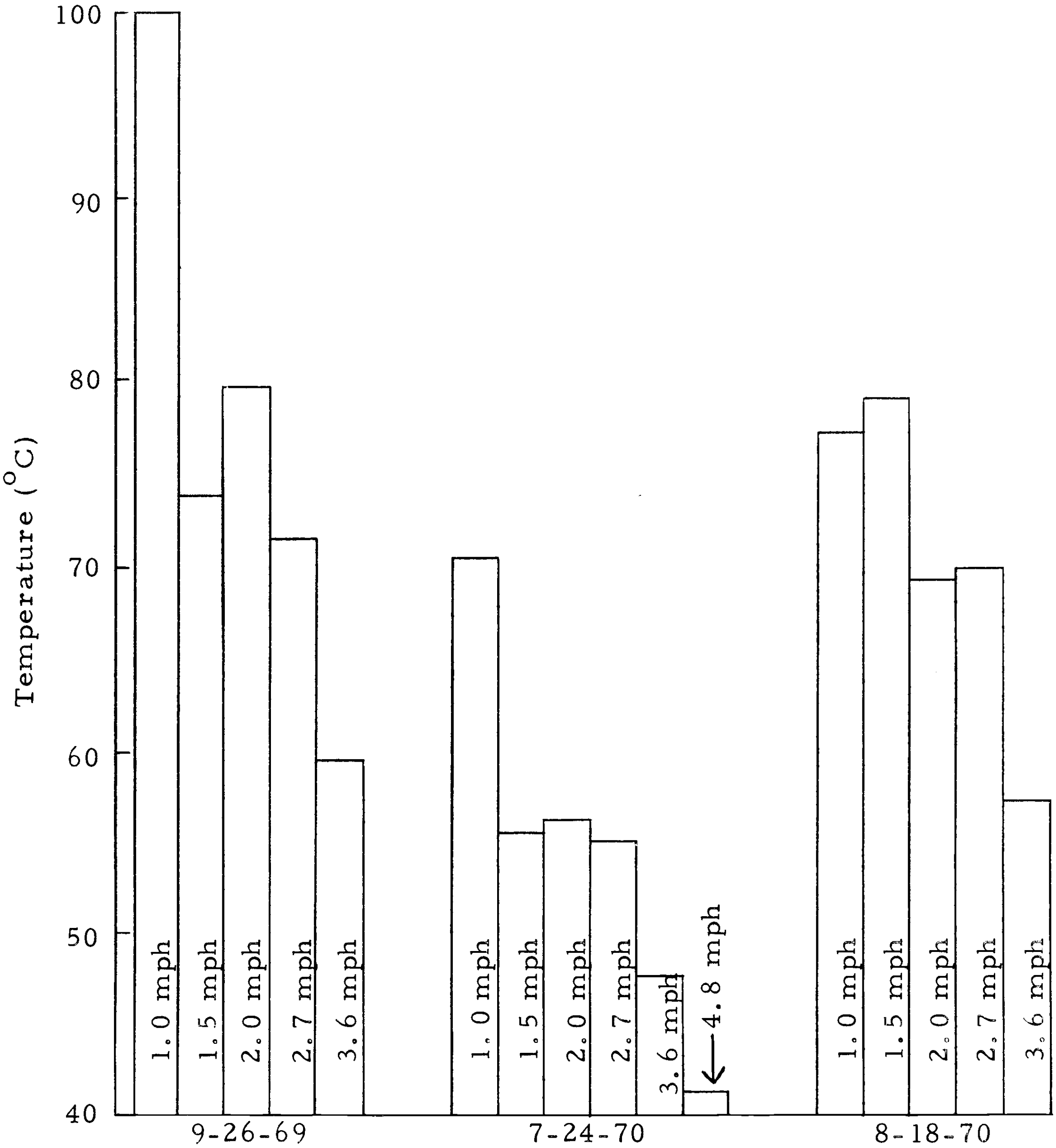

Figure 5. Speed versus internal stem temperature on three trial dates.

Table 4. Simple correlation coefficients for 77 observations from field flaming experiments.

Variables compared	Correlation Coefficient
$Speed^2$ to Speed	0.98**
$Speed^2$ to Peak Temperature	0.61**
$Speed^2$ to Final Count	0.67**
$Speed^2$ to % Kill	0.68**
Speed to Peak Temperature	0.61**
Speed to Final Count	0.58**
Speed to % Kill	0.59**
Peak Temperature to Final Count	0.43**
Peak Temperature to % Kill	0.46**

**Significant at the 1% level.

Nine variables were used for the regression analysis. These were:

1. Day one (September 26, 1969)
2. Day two (July 24, 1970)
3. Day three (August 18, 1970)
4. $Speed^2$
5. Speed
6. Initial count
7. Peak temperature
8. Final count
9. Percent kill

Ten different cases were examined by the multiple regression analysis. The cases were:

1. Final count versus days 1-3, $speed^2$, speed, initial count, and peak temperature.
2. Final count versus $speed^2$, speed, initial count, and peak temperature.
3. Percentage kill versus days 1-3, $speed^2$, speed, and peak temperature.
4. Percentage kill versus $speed^2$, speed and peak temperature.
5. Peak temperature versus days 1-3, $speed^2$, and speed.
6. Peak temperature versus $speed^2$, and speed.
7. Final count versus days 1-3, initial count, and peak

temperature.

8. Final count versus initial count and peak temperature.

9. Percentage kill versus days 1-3, and peak temperature.

10. Percentage kill versus peak temperature.

Table 5 illustrates how the computer fitted the data to the equation which this multiple regression analysis was generating. It also shows the order in which each variable entered into the equation and its corresponding F value.

Though peak temperature appeared to have some effect on the final count and on percentage kill, speed and speed^2 had a much greater effect on these two variables (Tables 4 and 5). Because peak temperature was not as highly correlated with percentage kill as was speed, the entire temperature curve over time was constructed and examined (Figure 6). Appendix Table 2 shows the temperature changes with time for all 77 observations. The temperature levels studied were time above 45, 50, 55, 60, 65, and 70C. Data were deleted from this analysis if there was zero time above the temperature level being correlated to the percentage kill of the fungus. Table 6 shows the simple correlation coefficients determined by this analysis.

Only time above 45 and 70C had any effect upon the percentage kill of <u>Verticillium</u>. The time above other temperatures did not appear to effect the percentage kill of the fungus.

Table 5. Variable entry and F values for multiple regression analysis of ten cases.

Case	Variable		Variable	F value
1	Final count	versus	Speed^2	62.50**
			Speed	29.47**
			Day 2	4.08**
			Day 3	0.75
			Initial count	2.97
			Peak temperature	0.04
2	Final count	versus	Speed^2	62.50**
			Speed	29.47**
			Peak temperature	1.19
			Initial count	0.01
3	Percentage kill	versus	Speed^2	65.90**
			Speed	30.20**
			Day 2	6.93**
			Day 3	0.93
			Initial count	0.01
4	Percentage kill	versus	Speed^2	65.90**
			Speed	30.20**
			Peak temperature	2.05
5	Peak temperature	versus	Day 2	47.14**
			Speed	57.28**
			Day 1	7.15**
			Speed^2	1.22
6	Peak temperature	versus	Speed	45.25**
			Speed^2	0.18
7	Final count	versus	Peak temperature	17.69**
			Day 3	1.56
			Initial count	0.45
			Day 2	0.06
8	Final count	versus	Peak temperature	17.69**
			Initial count	0.14
9	Percentage kill	versus	Peak temperature	20.19**
			Day 3	2.15
			Day 1	0.12
10	Percentage kill	versus	Peak temperature	20.19**

** Significant at the 1% level.

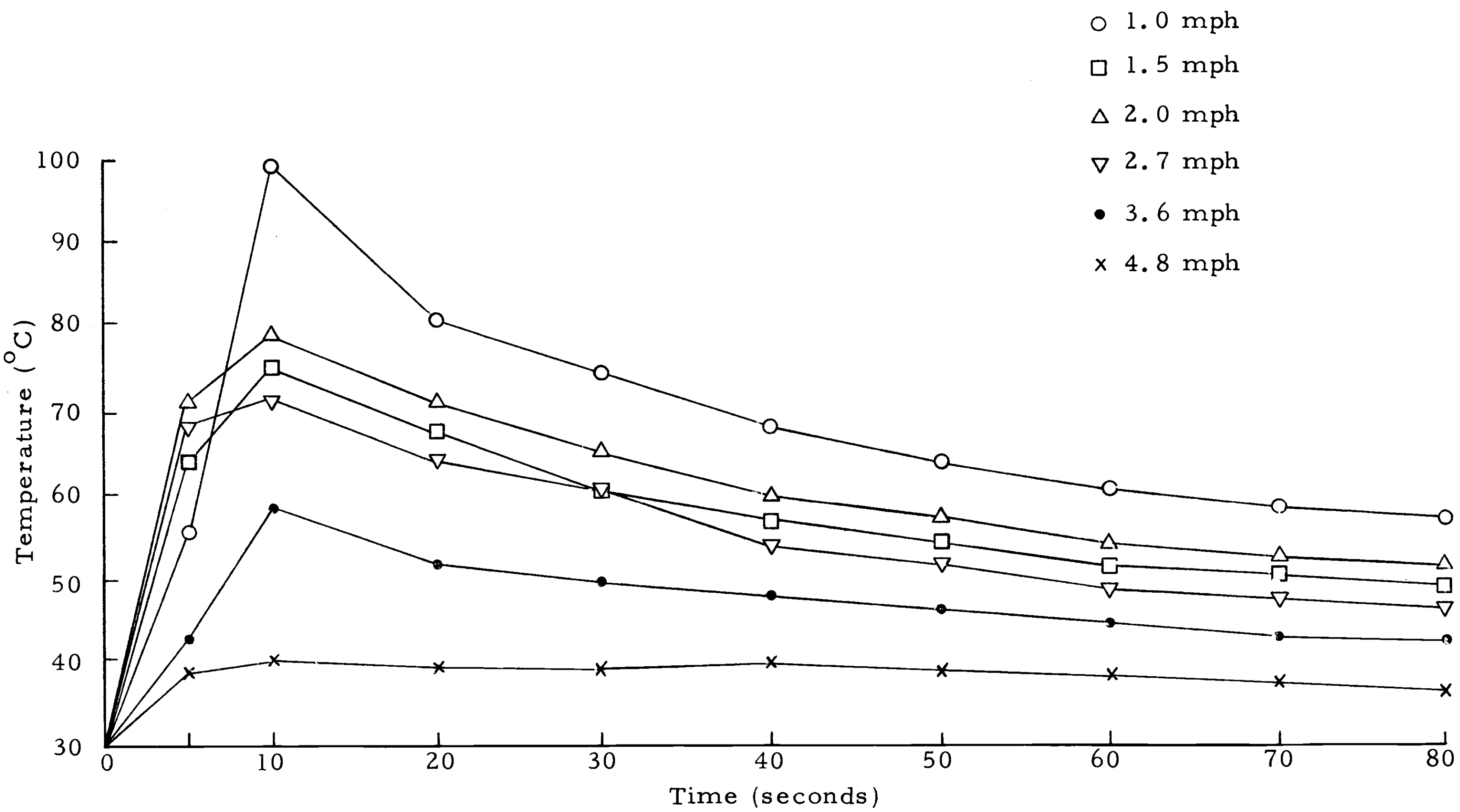

Figure 6. Typical internal temperature changes during an 80 second time period.

Table 6. Simple correlation coefficients for time above 45, 50, 55, 60, 65, and 70C with percentage kill of Verticillium.

Temperature level (^{o}C)	Number of variables	Simple Correlation coefficients
45	75	0.54**
50	69	0.03
55	62	-0.05
60	52	0.11
65	46	-0.24
70	37	-0.47**

*Significant at the 1% level.

Residual Assay

The 80 peppermint stems used in this experiment were propane flamed at a speed of 2.0 mph. The percentage kill was somewhat lower than expected. Average kill was 77.6% and ranged from 16.5 to 100% (Table 7). It can be noted that Verticillium increased in only one stem and was detected in only two other stems.

Because of the large number of saprophytes which invaded the stems as they lay in the field, it was difficult to determine positively that no Verticillium was present because the saprophytes could be masking its presence. During the final assay (November 24, 1970), stems 37 and 38 were prepared so that a low population of Verticillium could be detected. No Verticillium was observed using the modified assay.

Table 7. Survival of residual populations of Verticillium dahliae in stems placed in the field after flaming. (tractor speed = 2.0 mph, gas pressure = 35 psi)

Assay Date	Stem no.	% Kill after flaming	Number of propagules per gram tissue left after flaming	Propagules detected at assay	% Increase in Verticillium propagules	% Decrease in Verticillium propagules
7-28-70	1	75.4	44,320	0	-	100
	2	99.9	200	0	-	100
	3	97.6	36,960	0	-	100
	4	80.8	80,000	7,480	-	90.7
	5	95.4	34,800	0	-	100
8-4-70	6	100	0	0	-	100
	7	90.9	64,960	0	-	100
	8	16.5	50,080	0	-	100
	9	77.3	46,320	0	-	100
	10	92.6	5,400	0	-	100
8-11-70	11	96.6	8,080	0	-	100
	12	85.3	12,080	0	-	100
	13	85.4	58,000	0	-	100
	14	92.1	40,040	0	-	100
8-18-70	15	90.0	80,000	1,210	-	98.5
	16	52.3	80,000	0	-	100
	17	25.3	80,000	0	-	100
	18	77.7	78,960	0	-	100
	19	89.4	36,640	0	-	100

(Continued on next page)

Table 7. (Continued)

Assay Date	Stem no.	% Kill after flaming	Number of propagules per gram tissue left after flaming	Propagules detected at assay	% Increase in Verticillium propagules	% Decrease in Verticillium propagules
8-25-70	20	66.8	80,000	0	-	100
	21	87.7	25,840	0	-	100
	22	48.0	59,040	0	-	100
	23	26.3	24,760	0	-	100
	24	43.4	100,000	436,658	436.7	-
9-8-70	25	82.4	19,400	0	-	100
	26	66.9	80,000	0	-	100
	27	46.1	18,960	0	-	100
	28	98.3	5,960	0	-	100
	29	87.6	48,560	0	-	100
9-22-70	30	99.3	1,610	0	-	100
	31	96.3	560	0	-	100
	32	57.5	120,000	0	-	100
	33	89.2	80,000	0	-	100
	34	95.7	10,880	0	-	100
10-24-70	35	84.9	65,760	0	-	100
	36	82.0	47,360	0	-	100
	37	39.3	22,800	0	-	100
	38	89.4	38,280	0	-	100

Temperature Changes Under the Soil Surface

Temperature changes in the soil resulting from flaming were measured to determine the magnitude of change and to get some idea of their effect on mint rhizomes. Temperatures were measured at depths of 0.1, 0.2, 0.5, and 1.0 cm below the soil surface at flamer speeds of 2.0 and 2.5 mph; two runs were made at each speed for each depth (Table 8). At 2.0 mph, the temperature increased 11.8C at 0.1 cm below the soil surface and only 1.0C at a depth of 1.0 cm. An almost perfect inverse-square relationship between depth and and the temperature change at the depth was observed (Figure 7).

Table 8. Temperature changes induced in the soil by flaming.

Speed (mph)	Depth (cm)	Average temperature change (°C)	Average lag time until peak temperature attained (sec.)
2.0	1.0	1.0	80
	0.5	2.1	70
	0.2	7.3	45
	0.1	11.8	15
2.5	1.0	0.3	85
	0.5	1.0	80
	0.2	5.0	50
	0.1	8.0	20

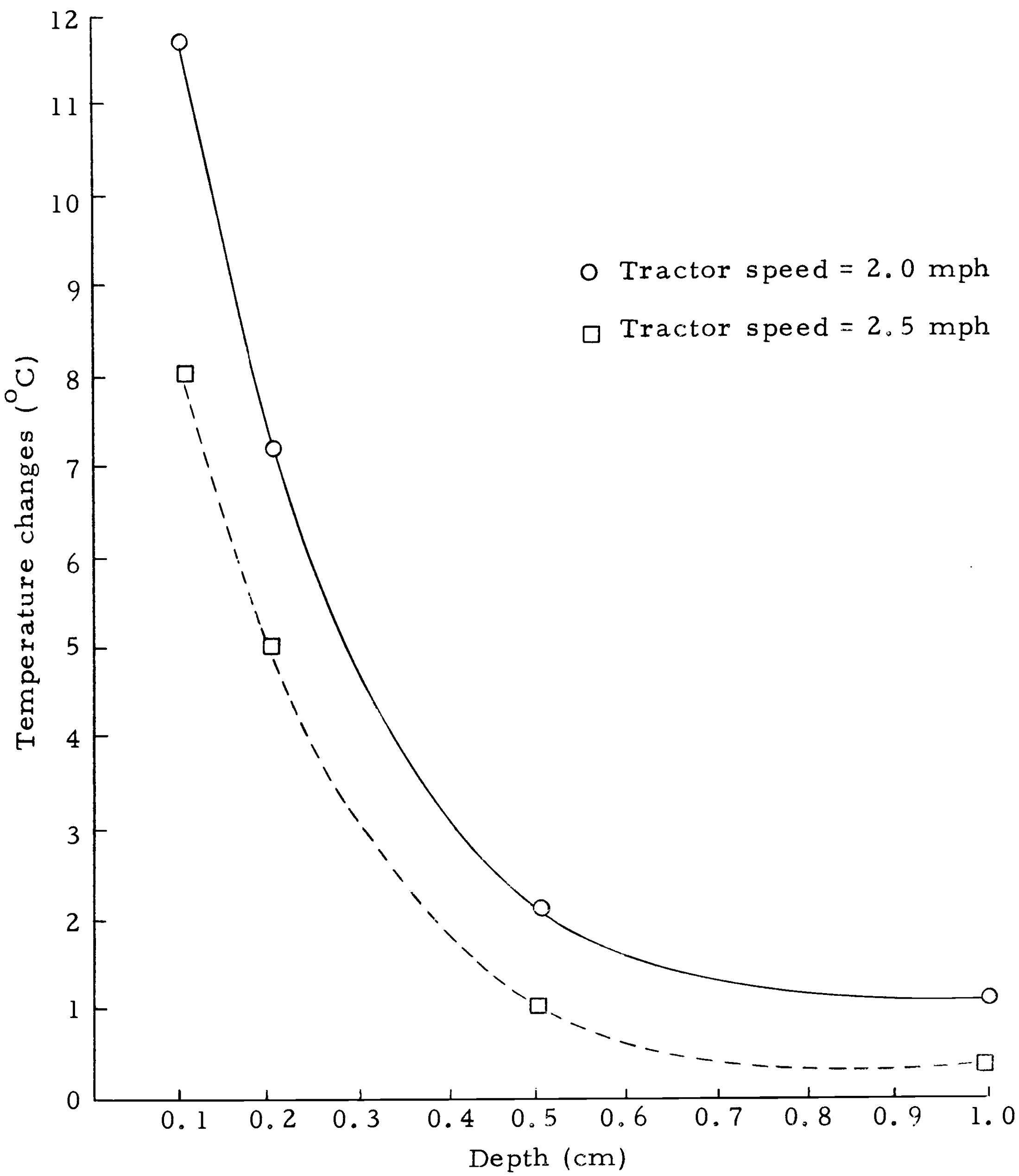

Figure 7. Average temperature changes at various soil depths.

DISCUSSION AND CONCLUSIONS

From limited published information (Horner and Dooley, 1965; Powelson and Gross, 1962), it was hypothesized that about 95% kill of Verticillium dahliae in diseased peppermint stems would be required to give adequate control of the fungal population. The preliminary laboratory flaming experiments indicated the internal stem temperatures required to obtain a 95% reduction in the Verticillium population. Forty degrees centigrade was too low, giving an average kill of only 35.0%. Fifty degrees centigrade gave an average kill of 83.0% and a range from 58.3 to 99.9% kill. The upper limit of this range substantially reduced the fungal population while the lower extreme gave too little kill. Sixty degrees centigrade reduced the population drastically, giving an average kill of 97.6% and a range from 94.3 to 100%. Temperatures at any higher level would, of course, adequately reduce the fungal population.

The preliminary laboratory experiments on population distribution showed that peppermint stems had a fairly even distribution of Verticillium. This allowed the percentage kill caused by flaming to be determined fairly accurately. Results from both preliminary experiments provided the basic information needed to conduct and analyze field tests.

The multiple regression analysis of data obtained from the field experiments showed that speed[3] was the most important variable determining percentage kill[4] of *Verticillium*. Speed was also correlated with peak temperature. Therefore, though peak temperature was not the best independent variable determining percentage kill, it was important because of its correlation with speed. In cases 7-10 of the multiple regression analysis (Table 5), where speed was eliminated as an independent variable, peak temperature affected the percentage kill of the fungus, and is therefore an important factor in reducing the fungal population.

Because peak temperature did not correlate with percentage kill of *Verticillium* as much as was expected, simple correlation coefficients were determined for the relationship of percentage kill and length of exposure above 45, 50, 55, 60, 65, and 70C. These simple correlation coefficients (Table 6) were vague in their meaning, but it appeared that time above 45 and 70C did have an effect on percentage

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[3]Both speed and speed^2 were used as variables in the multiple regression analysis because from observing the data it was obvious that there was not a straight line or polynomial relationship between speed and the variables of temperature and percent kill, but rather a combination of the two relationships. Although speed^2 correlated better with the other variables than did speed, both were significant at the 1% level. For ease of presentation, only speed will be used in the discussion.

[4]Both percentage kill and final count were used as variables in the multiple regression analysis. Because percentage kill correlated better with the other variables than did final count, it will be used in the discussion.

kill of the fungus, but that time above any other temperature level had little effect on percentage kill. Though some correlation is shown in this analysis, it is not biologically valid because it shows that the longer the temperature is above 70C, the less kill of the fungus. Because most data points gave percent kill at 95% or above, there was not much slope in the line connecting these points, and therefore, the simple correlation coefficients were either near zero or meaningless. The data which did show correlation was caused by a line connecting the few points which showed a low percentage kill with the large number of points which showed a high percentage kill. Because of a lack of points between these two extremes, no definite conclusions should be made from these observations.

Internal stem temperature did not correlate as highly as did speed with percentage kill because most of the error was associated with stem temperature values. The diameter and maturity of the peppermint stem would affect the internal stem temperature. Positioning of the probe in the stem would also introduce variation. The heat from the propane flame is highly variable under the hover (Perumpral, Lien and Liljedahl, 1966). If the probe happens to be at a "cooler" or "hotter" spot during different runs, the observed temperature could be quite misleading. These factors, and possibly more, could explain why speed, which is a regulated variable, is more closely correlated with percentage kill than is temperature.

At speeds from 1.0 to 2.7 mph only one stem assayed had less than 95% kill of Verticillium (Appendix Table 1). At higher speeds, variability in percentage kill of the fungus increased. Speeds should be kept at 2.0 or 2.5 mph to obtain an average kill near 95%. Another reason to use speeds of 2.0 to 2.5 mph is that an important function of propane flaming is to incinerate the dried leaf and stem debris which is on the ground (Horner and Dooley, 1965). I observed that this trash, which contains Verticillium, is incinerated at speeds of 2.5 mph or less, but at higher speeds much of it may remain unburned. The burning of this debris eliminates a source of inoculum that otherwise would function in spread and increase of disease severity. Therefore, the total function of propane flaming would be to 1) eliminate the wilt fungus in dead plant debris by incineration, and 2) drastically reduce the fungal population in the erect, live stubble by heat inactivation (thermotherapy).

The multiple regression analysis also suggests that the time of flaming may be important. Cases 5 and 6 (Table 5) suggest that peak temperature is influenced most by the day and speed. It is not always possible to control the day the flaming will be done, but the speed at which it is done can be altered to the conditions of the day. The equation $H = Ms(t_2 - t_1)$ (Semat, 1963) where H = energy input, M = mass, s = specific heat, and t_1 = initial and t_2 = final temperatures, shows that the initial internal stem temperature will influence the final internal stem temperature if a constant amount of energy is put into the system. Therefore, one must go slower on cool days than on warmer days. Reducing speed on cool days will increase the energy put into the system so that the peak temperature obtained will be equal to the peak temperature obtained on warmer days at faster speeds. Therefore, on cool mornings or days, it is best to flame at 2.0 mph while on warmer days the speed may be safely increased to 2.5 mph.

It also appears important to avoid flaming when the ground is very wet. Unpublished data provided by Glenn Page, Agricultural Engineer at Oregon State University, shows that much of the energy generated by the propane flamer on wet surfaces goes into the formation of steam. This immediately lowers the external stem temperature to a maximum of only 100C until the free water is vaporized. Therefore, internal stem temperature changes would probably be reduced, thereby reducing the percentage kill of Verticillium. It has been noted (Horner and Dooley, 1965) that in dry versus wet field conditions, the percentage kill of the fungus is much less in wet than in dry field conditions.

The results of the residual assays, showing that little or none of the residual population of Verticillium remains one week after flaming, were surprising. Perhaps the fungal population present immediately after flaming was injured and slowly died from heat shock. Another possible explanation is that V. dahliae, which is in its vegetative, parasitic stage at the time of flaming, cannot adjust rapidly enough to form its saprophytic or dormant stage, and therefore perishes.

In the residual population experiment[5], three of 38 stems assayed contained V. dahliae (Table 7). It appeared that the percentage kill of the fungus by flaming did not determine whether or not a residual population survived. Instead, the size of the population left after flaming may be an important factor. In all instances where there was a residual population there were at least 80,000 Verticillium propagules per gram tissue left after flaming. Ten of the 38 flamed stems had a population near or above 80,000 propagules per gram after flaming. The three with a residual population were in this group. Two of these three had a population decrease of at least 90%, while one showed an increase of 436%. The results show that: (1) most flamed stems carry an initial residual population; (2) most of the initial residual population dies soon after flaming; (3) those stems with a very high population after flaming are the ones most likely to carry a surviving residual population; and (4) only rarely will there be an increase in the surviving residual population.

Because of the high population of Verticillium per gram of tissue, it is important to attain a high percentage kill, possibly 95%.

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[5] The residual assay experiment is continuing at this time on the 42 stems remaining in the field.

This will reduce the incidence of stems containing more than 80,000 propagules per gram tissue after flaming. If the speed was increased so that 95% kill was not obtained, there could be a large buildup of the fungus even if very few stems contained more than 80,000 propagules per gram after flaming. This is because at higher speeds, lower internal stem temperatures would occur and there would not be as much injury to the peppermint stems or fungus. This could allow an increase in the residual population where it existed in numbers much less than 80,000 propagules per gram.

Weather conditions after flaming might have an effect upon survival of the residual population. Weather during the first five residual assays was generally dry and warm, and three of 24 stems showed a residual population. Had the weather been cool and moist, the survival rate might have been better. However, before each of the last three assays, it rained hard for several days, yet none of 14 stems had a residual population. Seven of the ten stems with a population of 80,000 propagules per gram after flaming were observed during the first five assays, and the other three were observed during the last three assays. This again seems to indicate that the most important factor in determining if there will or will not be a surviving residual population is whether a high population of *Verticillium* is left after flaming.

Peppermint produces both stolons and rhizomes. Because most of the stolons above the soil surface do not survive normal winter conditions, what occurs to them during flaming is of little significance. The rhizomes which are below the soil surface are important for regrowth of the peppermint the following spring. The data show that at 2.0 mph there was a 1.0C change at a depth of 1.0 cm, and at 2.5 mph there was a 0.3C change at the same depth (Table 8). These temperature changes caused by flaming are of no consequence to the rhizomes at this depth, and rhizomes at lesser depths should also stand a good chance of survival. Therefore, flaming at 2.0 or 2.5 mph is of little or no consequence to a future peppermint crop.

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APPENDIX

Appendix Table 1. Peak temperature and percent kill of Verticillium on three separate dates and six different tractor speeds (gas pressure = 35 psi).

Speed (mph)	Date	Peak temperature (°C)	Percent kill
1.0	9-26-69	100	95.8
		100	99.6
		100	99.6
	7-24-70	75	99.9
		66	100
		70	99.9
		77	99.4
	8-18-70	67	97.7
		68	100
		90	100
		80	100
		87	98.4
1.5	9-26-69	75	99.5
		80	98.5
		70	99.1
		60	100
		100	99.9
		72	99.9
		79	98.1
	7-24-70	50	100
		49	100
		63	98.2
		66	99.2
		54	99.9
	8-18-70	69	98.8
		79	100
		79	99.9
		89	99.9
		79	100
2.0	9-26-69	79	99.9
		95	99.9
		80	98.7
		95	99.9
		73	98.4
		80	99.1

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Appendix Table 1. (Continued)

Speed (mph)	Date	Peak temperature (°C)	Percent kill
2.0	7-24-70	62	100
		52	98.9
		55	99.6
		55	99.9
		55	100
	8-18-70	70	100
		64	100
		75	100
		63	100
		81	100
2.7	9-26-69	74	99.8
		76	99.4
		95	99.9
		61	69.2
		65	99.3
	7-24-70	57	99.1
		52	99.3
		56	100
		56	100
		55	99.8
	8-18-70	69	95.3
		72	99.0
		68	99.9
		70	99.9
		70	99.1
3.6	9-26-69	46	99.8
		60	96.5
		70	98.9
		65	98.1
	7-24-70	50	95.9
		49	47.2
		46	23.2
		49	100
		46	69.3

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Appendix Table 1. (Continued).

Speed (mph)	Date	Peak temperature (°C)	Percent kill
3.6	8-18-70	53	99.7
		70	100
		55	100
		58	95.4
		58	100
4.8	7-24-70	41	18.0
		45	19.9
		39	17.0

Appendix Table 2. Internal stem temperature changes over an 80 second time period on three different dates and at six different speeds (gas pressure = 35 psi).

Date	Speed (mph)	Time intervals (sec.) 0	5	10	20	30	40	50	60	70	80
		°C	°C	°C	°C	°C	°C	°C	°C	°C	°C
9-26-69	1.0	31	56	100	80	72	66	62	58	55	52
		32	59	100	78	71	65	62	58	55	52
		33	64	100	81	74	68	63	59	55	53
7-24-70		34	69	75	63	60	56	53	50	48	46
		36	66	60	63	60	57	54	53	51	50
		34	70	60	65	62	60	57	54	52	51
		35	73	77	67	63	59	56	53	51	50
		36	71	80	64	59	57	54	51	48	47
8-18-70		35	45	67	63	58	54	51	48	46	44
		36	55	68	64	60	56	53	50	48	46
		36	60	90	78	68	62	58	53	50	48
		37	70	80	75	63	56	54	52	50	48
		40	87	82	72	63	56	53	52	50	48
9-26-69	1.5	28	40	75	60	55	53	51	47	45	42
		29	65	80	71	63	58	55	52	49	45
		31	65	70	69	56	51	47	44	41	40
		32	55	60	55	52	49	47	44	43	41
		33	100	80	66	60	57	53	51	50	51
		28	60	72	65	58	52	49	45	43	40
		31	69	79	74	65	60	56	53	50	47

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Appendix Table 2. (Continued)

Date	Speed (mph)	Time intervals (sec.) 0	5	10	20	30	40	50	60	70	80
		°C	°C	°C	°C	°C	°C	°C	°C	°C	°C
7-24-70	1.5	30	45	50	48	46	44	43	41	41	40
		33	46	49	47	46	45	44	43	42	42
		36	55	63	60	57	55	53	52	50	49
		35	66	61	56	52	49	47	44	43	41
		32	50	54	51	49	47	46	45	44	43
8-18-70		38	60	69	64	59	55	52	50	48	47
		39	74	79	67	59	53	50	47	45	44
		37	44	79	66	59	55	52	50	48	47
		38	80	89	67	57	51	47	46	44	44
		37	70	79	69	62	57	52	50	48	47
9-26-69	2.0	31	65	79	66	59	53	49	46	44	42
		31	95	80	70	59	53	48	44	41	39
		32	55	80	75	67	60	55	50	47	44
		33	95	87	74	65	59	55	51	48	46
		32	65	73	68	61	57	53	50	47	45
7-24-70		36	60	62	57	53	51	49	47	46	45
		34	50	52	51	49	48	46	45	44	44
		33	53	55	53	51	50	48	47	46	45
		35	50	55	54	53	51	50	49	48	47
		34	50	55	54	53	52	51	50	49	48

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Appendix Table 2. (Continued)

Date	Speed (mph)	Time intervals (sec.)									
		0	5	10	20	30	40	50	60	70	80
		°C	°C	°C	°C	°C	°C	°C	°C	°C	°C
8-18-70	2.0	38	69	70	62	57	53	50	48	47	46
		37	64	60	59	56	53	50	48	47	46
		38	71	75	63	55	51	48	45	43	42
		38	63	61	58	53	49	47	45	43	42
		37	74	81	61	52	48	43	41	41	41
9-26-69	2.7	32	65	74	64	58	54	51	49	47	45
		32	65	76	67	59	55	51	49	47	45
		31	95	80	72	62	55	51	47	45	43
		29	50	61	58	55	50	47	45	43	41
		31	55	65	61	56	52	48	46	44	42
7-24-70		36	55	57	54	51	50	49	47	45	44
		33	50	51	50	48	46	45	44	42	41
		34	54	56	54	52	49	47	45	44	42
		36	55	56	54	52	50	49	48	47	46
		36	54	55	53	52	50	49	48	45	44
8-18-70		37	67	69	60	53	49	47	45	43	42
		37	69	72	61	54	50	47	45	43	42
		38	63	68	55	49	47	44	42	41	41
		38	66	70	59	53	50	47	45	43	42
		37	69	70	60	55	50	49	47	45	43

(Continued on next page)

Appendix Table 2. (Continued)

Date	Speed (mph)	Time intervals (sec.)									
		0	5	10	20	30	40	50	60	70	80
		°C	°C	°C	°C	°C	°C	°C	°C	°C	°C
9-26-69	3.6	32	41	47	46	45	44	44	43	43	42
		30	55	60	55	51	48	45	43	41	40
		31	70	65	58	52	48	45	42	40	39
		31	50	65	54	49	45	43	41	39	37
7-24-70		34	50	49	47	46	45	44	43	42	41
		36	49	48	46	45	44	43	42	41	40
		34	44	45	44	44	43	42	42	41	41
		31	47	49	48	47	46	45	44	43	42
		36	45	46	45	44	43	42	41	40	39
8-18-70		38	52	53	52	50	48	47	45	44	43
		38	70	64	56	51	47	45	43	42	42
		38	55	54	51	48	47	45	43	42	41
		38	49	58	46	44	43	43	42	42	42
		39	58	56	55	52	50	49	48	47	46
7-24-70	4.8	34	37	41	40	40	40	39	39	39	39
		36	40	45	43	41	40	40	39	39	39
		35	35	39	38	38	38	38	38	38	37