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Title A DISEASE OF PEPPERMINT CAUSED BY PHOMA MENTHAE

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Phoma menthae Strasser causes black lesions and cankers on stems and rhizomes of Mentha piperita L. The fungus was isolated from infected rhizomes and stems.

The optimum temperature for growth of the fungus in culture was between 20-25°C; little growth occurred at 5°C, and no growth occurred at 35.5°C.

Among seven carbon sources tested in liquid culture, Phoma menthae grew best on starch and poorest on glucose and maltose. The fungus grew well with fructose or galactose as carbon sources.

Inoculation of healthy peppermint stems with or without wounds, produced typical symptoms of the disease. Disease developed most rapidly, however, in plants inoculated in wounds. Dry harvest weights of plants inoculated in wounds were greatly reduced as compared with those inoculated without wounds. Phoma menthae was reisolated from infected plants.

Root inoculation reduced greatly the dry harvest weights of inoculated plants as compared with controls. The fungus was re-isolated from roots of stunted plants.

The disease developed rapidly on peppermint rhizomes between 19-25.1°C; no disease developed at 30.1°C.

Plants inoculated at different ages demonstrated that resistance to the action of the fungus increased with plant age.

A DISEASE OF PEPPERMINT CAUSED BY PHOMA MENTHAE

by

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A DISEASE OF PEPPERMINT CAUSED BY *PHOMA MENTHAE*

INTRODUCTION

A disease of peppermint, characterized by black lesions and cankers on stems and rhizomes, and red color on the top leaves of the plant, occurs in both field and greenhouse grown peppermint in Oregon.

A fungus of the genus *Phoma* was isolated from diseased material. The purpose of this study was to: 1) identify the *Phoma* sp., 2) determine if the *Phoma* in question was the causal agent, 3) learn something about growth of the fungus in culture and the effects of temperature on growth and on disease development, 4) learn something about the host-parasite relationship through establishment of infection in healthy plants by various inoculation methods, and 5) learn how plant age affects disease severity.

LITERATURE REVIEW

Most Phoma species were described on the basis of their occurrence on a single host plant species. During the period of descriptive mycology (about 1850 - 1930), finding Phoma on a new host was automatically the occasion for describing a new species. Hundreds of the described species of Phoma and Macrophoma cannot be separated morphologically, but no modern mycologist has yet attempted to revise the genus.

The disease caused by Phoma menthae on peppermint (Mentha piperita L.) is similar to diseases caused by various Phoma spp. on several other crop plants. This review of literature will consider mostly those diseases caused by Phoma spp. which can be characterized generally as stem rots, stem cankers, or root rots.

In 1910, Phoma menthae was described by Strasser on Mentha silvestris in Austria (12). A translation of the description as it appears in Saccardo (Vol. 22, p. 882) is as follows: "Pycnidia subspherical, covered by the epidermis, ostiole prominent, brownish, 200-280μ in diameter; spore masses pinkish; spores single-celled, hyaline, elongate to ellipsoid to ovoid, 4-5 X 3-3.5, biguttate. Habitat: in stems of Mentha silvestris, Sonntagberg, Austria."

A search of the literature has revealed no other description of Phoma menthae or disease attributed to it. Nearly 2000 species

of Phoma and Macrophoma have been described (17).

Spring black stem of alfalfa is caused by Phoma herbarum var. medicaginis. The disease is widespread and may cause considerable reductions in yield under cool, moist conditions. This disease may affect all parts of the plant, but usually the most striking symptoms are the black, elongate lesions on the stem (13).

Phoma herbarum var. medicaginis was isolated from 96% of the blackened stems of alfalfa from 13 north-central states of the United States of America during six years. The causal fungus was most pathogenic at 18°C (8).

Alfalfa was successfully inoculated with P. h. var. medicaginis by spraying with conidial suspensions. Infection was proportional to conidial concentration. When alfalfa leaves were wounded by blowing carborandum on them with DeVilbiss aspirator, lesions were more numerous on wounded plants than on those unwounded, regardless of the amount of inoculum applied. Under field conditions older alfalfa plants are more heavily infected than seedlings, possibly because of a gradual build-up of inoculum as well as increased susceptibility (1).

Successful leaf inoculations of alfalfa were made in the greenhouse by spraying leaves with concentrated spore suspensions of P. h. var. medicaginis. Stems were successfully inoculated by applying spore suspensions with a camel hair brush to a small

section of the stem, which was then wrapped with aluminum foil. Growth of the fungus usually was confined to the cortical tissues of the stem; it did not enter the vascular tissue. Pycnidia developed in dead cortical tissues beneath the epidermis of the stem and ruptured the epidermis as they reached maturity. The optimum temperature for growth of P. h. var. medicaginis on potato dextrose agar from mass transfer was between 20-25°C; the maximum at which growth occurred was 30°C; no growth developed at 35°C.

The pustule disease of flax, caused by Phoma lini is characterized by browning and drying of the leaves and breaking of the stems at the base (10).

P. lini is common on flax in Germany where it produces a dense coating of dark brown, bulbiform to spherical pustules, mostly measuring 0.15 to 0.35 by 0.12 to 0.27 mm., from the stem base up to a height of 10 cm. The pathogen causes wilting and premature defoliation, while pycnidia are formed in masses on the stems. The organism was isolated from diseased flax stems on potato juice agar plates at 25°C. The effect of temperature on linear growth of the organism on potato juice agar was: minimum 3°C, maximum 30°C, and optimum 25°C (9).

Infection by P. lini was obtained most rapidly by inoculating plants with small pieces of agar culture after scraping off the waxy cuticle. It was presumed that infection by this fungus was possible

only if plants were first injured by nymphs of Thrips lini. Experiments, however, indicated that seedlings contracted infection from the soil. Under apparently uniform conditions some plants among the infected ones remained healthy (10).

Phoma lini, through the operation of its cellulolytic enzymes, could disorganize the natural cellulose of flax and other forms of cellulose and hemicellulose (11).

Phoma lingam caused losses of 80 to 100 percent in soil inoculation tests on flax (9).

Phoma lingam is the causal organism of blackleg disease of Crucifers. This disease may affect cabbage, cauliflower, broccoli, kohlrabi, kale, brussels sprouts, turnip, rutabaga, and Chinese cabbage. Symptoms on cabbage and other members of the cabbage family occur first in the seedbed two or three weeks before transplanting. Spots appear on the leaves as inconspicuous, indefinite pallid areas that gradually become well-defined spots with ashen-gray centers in which the pycnidia are scattered irregularly. On stems the spots are more linear and are often surrounded by purplish borders. Stem lesions at the soil line usually extend to the root system, causing dark cankers in which pycnidia are less common until after the plant is dead. On rutabaga and turnip seedlings, lesions are like those on young cabbage. As the fleshy roots form, they are also affected either before harvest or after

they are placed in bins or pits. Infection is followed by a canker of growing roots and a dry rot in storage. The surface of the decayed tissue may bear pycnidia in abundance (16, p. 289).

Phoma lingam cause dry rot in field grown and canker in stored cabbage in the Netherlands. Symptoms of dry rot appear after transplanting as a leaden discoloration of the leaves; the death of the tap-roots results in decay of the plants. Canker develops only during storage, when the fungus penetrates from vascular bundles into the pith and cortex, forming dark sunken lesions. Diseased seed is the principal source of infection. P. lingam may persist in infected seed for as long as ten years (15).

A note-worthy disease of kohlrabi is caused by Phoma lingam. Oval spots with a blue-green rim appear on the swollen part of the stem, the centers becoming dry, brittle, and torn, and the tissues up to 0.5-1 cm. underneath are gray-black and partially rotten. Abundant pycnidia developed on the dead tissue (14).

Celery scab, caused by Phoma apiicola, is a source of heavy losses to German growers. The disease occurs in a particularly severe form after a cool, damp summer. In the greenhouse, inoculation of seedlings in sandy soil with a 40 percent moisture-holding capacity resulted in 100 percent infection. Celery seedlings were inoculated at transplanting with a spore suspension of the fungus in batches of 30 at four-day intervals from May until the middle of

September. At harvest the heaviest infection occurred on the roots of plants inoculated in May and June, decreasing thereafter to nil. It was concluded that the later transplanting is accomplished, the lower will be the incidence of scab. This assumption was confirmed by a test in which plants of the same age inoculated on May 10 and 23, June 14, and July 23 developed respectively 77, 38, 9, and 3.4 percent scab (4).

The optimum temperature for spore germination and mycelial growth of Phoma apiicola is 25°C (5).

A root rot of garden beets is caused by Phoma betae; the disease usually appears at the root tips but occasionally occurs at the crown and in wounds on the side of the root. The affected tissues blacken or develop a flat, grayish-white surface mycelium while internally the dark brown watersoaked tissues, sharply divided from the healthy tissues, become black and granular and eventually become dry and spongy. Cultures of Phoma betae isolated from garden beets grew on potato-dextrose agar (pH 7) through temperature ranges of 35° to 95°F. Optimum growth was obtained at 75°F. Wound inoculation in whole and sliced garden beets produced greater decay during storage in older beets than in young beets. Beets inoculated by the scalpel method and stored in moist sand for six weeks developed lesions averaging 5 mm. in diameter at 45°F, 12 mm. at 55°F, and 6 mm. at 65°F. Different amount of decay produced in sliced

storage beets inoculated with five different cultures of P. betae from garden beets and three from sugar beets, indicated that there may be some difference in pathogenicity between strains of the organism (6).

Phoma betae also causes a stem rot of beet seed plants. External symptoms appear at the end of July or early in August in the form of black necrotic lesions, 1 to 25 by 0.5 to 3 cm., mostly confined to the lower third of the stem in the early stages but tending later to coalesce and involve the upper part also. The pathogen is predominantly restricted to the outer tissues, seldom proceeding beyond the cambium into the xylem of the vascular bundles and medullary tissue. Inoculations with mono-ascospore cultures of Mycosphaerella tabifica (=Phoma betae) on healthy seed-beet stems resulted in the formation of typical brown, striate necrosis containing pycnidia (2).

Carrots in the Moscow region have been widely attacked by Phoma rostrupii, seedling and storage losses sometimes amounting to 95 percent. Optimum conditions for the development of the pathogen are high air and soil humidity, temperatures of 20-25^{o}C, pH 5.5-7, and diffuse light (3).

A root rot of Chrysanthemum morifolium "White Shasta", resulting in death of infected roots, general stunting, foliar chlorosis and necrosis is caused by a species of Phoma. Soil inoculations

with monoconidial cultures of the Phoma sp. found associated with infected plant roots reproduced the symptoms, which were most severe on plants growing in soil maintained at 55-60°F. Inoculation of the aerial parts of the plant were negative. The fungus caused mainly a cortical infection which was little affected by soil pH or change in soil humidity. There was no evidence that the fungus was carried in cuttings (7).

MATERIALS AND METHODS

Studies on the Causal Agent

Isolation of Phoma Menthae

Phoma menthae was isolated from infected rhizomes of peppermint (Mentha piperita L.), which were collected from plants grown in ground beds in the greenhouse at Oregon State University Agricultural Experiment Station. The organism was isolated on potato-dextrose agar medium containing 100 ppm streptomycin, transferred to Czapek Dox agar medium, and maintained on this medium at laboratory temperature of 23-25°C.

Temperature and Linear Growth in Culture

Effect of temperatures ranging from 5°C to 35. 5°C on growth of Phoma menthae on Czapek Dox agar medium (pH 7. 3 at 45°C) were made in flat-bottom petri plates containing 20 ml of medium. Agar was added to Czapek Dox solution at 17 gm per litre. The medium was sterilized at 15 pounds per square inch pressure for 20 minutes. After sterilization the medium was allowed to cool to 45°C, then the pH was measured by a Beckman Zeromatic pH meter. Twenty ml of medium was placed in each plate with a hand automatic pipette.

The averages of temperatures in the incubators were 5, 10, 15.5, 18.7, 25, 31, and 35.5°C. The petri plates were inoculated in the center with discs 4 mm. in diameter of Czapek Dox agar medium on which the organism was grown for two weeks. Before inoculation, plates were brought to the temperature of each incubator by placing them in the incubators for two hours. The rate of growth was measured as radial growth in mm. For each temperature four replicates were made.

Growth on Various Carbon Sources

To determine the best carbon source for growth of Phoma menthae, the following were tested: glucose, fructose, galactose, maltose, lactose, sucrose, and starch. The basal medium contained the following per liter:

$NaNO_3$	3 gm
K_2HPO_4	1 gm
$MgSO_4 \cdot 7H_2O$	0.5 gm
KCl	0.5 gm
$FeCl_2 \cdot 4H_2O$	0.01 gm
Biotin	5 μg

Each carbon source was added to the basal medium to make a final concentration of 20 grams per litre. All carbon source media were sterilized by filtration except the starch which was sterilized as powder separately by autoclaving. The starch was

placed in a container which contained calcium chloride to absorb moisture during autoclaving. Initial pH of the media was determined by a Beckman Zeromatic pH meter.

Phoma menthae was grown on the various carbon sources in 250 Erlenmeyer flasks, each containing 50 ml of medium. The media were inoculated with 1 ml of a spore suspension containing 200,000 spores per ml. The spore suspension was obtained by scraping spores from a culture grown on Czapek Dox agar and suspending them in distilled, sterilized water. The spores were washed twice by centrifuging and then re-suspended in distilled sterilized water. Four replicates of each carbon source were inoculated. Flasks were incubated under continuous light which was provided by flourescent tubes. The incubation temperature varied from 25 to 29°C.

Three harvests of the fungus were made: the first after 8 days, the second after 14 days, and the third after 18 days incubation. Dry weight of mycelia and spores was used as the criterion of growth. Mycelia and spores were removed from the media by filtration on pre-weighed fiberglass pads and then dried in an oven at 110°C for two hours. Final pH of media were measured at the time of each harvest.

Studies on the Disease

Test Plants

The plants used in these tests were obtained by placing tip cutting of mint (Mentha piperita L. var. mitcham) in sand flats. After 1-2 weeks the cuttings rooted, and each rooted cutting was transplanted to a pot filled with soil. The plants grew in soil for one week or more after transplanting before inoculation.

Methods of Inoculation

Four methods of inoculation were used: 1) inoculation of the stem without wounding, 2) inoculation of the stem with incision wounding, 3) inoculation of the stem after puncturing it with a needle, and 4) root inoculation. In the first three methods, the inoculum was placed about 3 cm above the soil level of the pots. The inoculum used was a piece of Czapek Dox agar medium on which the organism had grown for two weeks. After putting the inoculum on the infection court, the stem was wrapped at the inoculation point with cheesecloth and cellophane tape. With a pipette, two drops of distilled sterilized water were applied to the cheesecloth to keep the inoculation area moist. After inoculation the plants were kept on a greenhouse bench. Natural daylight was supplemented by flourescent tubes to provide a 16-hour day

length. Plants were watered every day and fertilized each week. The plants used in this test were about five inches high at the time of inoculation. For each of the first three methods of inoculation, ten plants were used. Twenty plants served as controls; ten were wounded but not inoculated, and ten were not wounded.

Root inoculation was accomplished by dipping rooted cuttings in a spore suspension containing 100,000 spores per ml; each plant was then transplanted to a pot of soil. Ten forty-day-old plants were used in this test and ten plants served as control. Plants were watered every day and fertilized each week. The symptoms were recorded; at the end of the experiment the dry weights of the plants were determined.

Temperature and Disease Development

The effect of six different temperatures on development of the disease on mint runners tips were tested. The average of temperatures used were 5°, 10.1°, 15°, 19°, 25.1°, and 30.1°C.

Glass test tubes 25 x 200 mm were filled one fifth full with white washed sand saturated with Hoagland's solution (about 8 ml per tube). The tubes were then plugged and sterilized in the autoclave for 15 minutes at 15 pounds pressure.

Runner tips about 15 cm long were collected from mint plants growing in the greenhouse. Runner tips were punctured near the

middle with a needle, then a small piece of Czapek Dox agar on which P. menthae had grown for two weeks was applied to the wound. After inoculation a runner was placed in each glass tube. The tubes were placed in incubators at different temperatures.

Eight runner tips were placed at each temperature; four served as non-inoculated controls. Length of lesions that developed after three, four, and five days of incubation at the various temperatures were measured and recorded. Figure 1 shows the method used and a lesion on a runner.

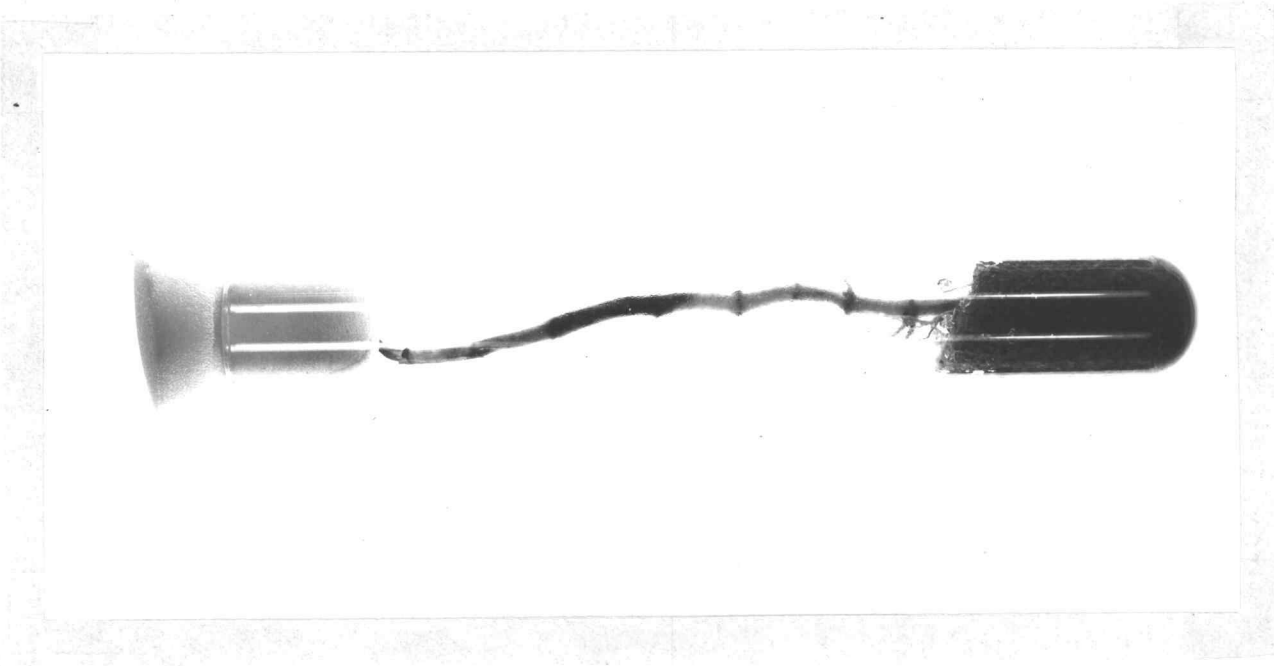

Figure 1. Peppermint rhizome tip growing in nutrient sand culture and inoculated with Phoma menthae.

Assessment of Disease Severity

Disease severity was assessed by a disease rating scheme. The numbers 0, 1, 2, 3, and 4 designated no disease, light disease,

moderate disease, severe disease, and death respectively. The number 0 designated plants which were healthy, 1 designated plants on which developed a small black lesion on the stems and red color on the top leaves, 2 designated plants on which elongate black lesions appeared followed by loss of lower leaves, 3 designated plants on which large lesions caused rapid collapse, and 4 designated plants which were dead. Figure 2 shows plants representative of the disease classes.

Figure 2. Peppermint plants inoculated with Phoma menthae and showing different classes of disease severity.

Dry harvest weights were used to determine disease severity. Plants were cut at the soil level and dried in a laboratory oven at 105°C for 48 hours, then weighed.

RESULTS

The Causal Agent

Effect of Temperature on Linear Growth in Culture

This experiment was made to test seven different temperatures ranging from 5°C to 35.5°C on growth of Phoma menthae in culture. The data are presented in Table 1 and shown graphically in Figure 3.

Table 1. Effect of temperature on radial growth of Phoma menthae.

Temp. C°	Radial growth in mm at indicated days after inoculation*				Average radial growth in mm/24 hrs.
	2	3	5	7	
5.0	-	-	4.3	6.3	1.0
10.0	+	4.6	10.4	16.2	2.9
15.5	7.3	11.5	21.0	31.5	4.8
18.7	9.1	14.5	25.6	37.3	5.6
25.0	11.1	17.5	30.6	44.1	6.6
31.0	+	4.5	4.9	5.3	0.2
35.5	-	-	-	-	-

*Average of 4 replicates; -, no growth; +, trace of growth.

The data show that P. menthae has an optimum temperature for growth between 20° and 25°C. Little growth occurred at 5° and 30°C, and no growth was observed at 35.5°C. At all temperatures tested, the organism made visible growth within three days after

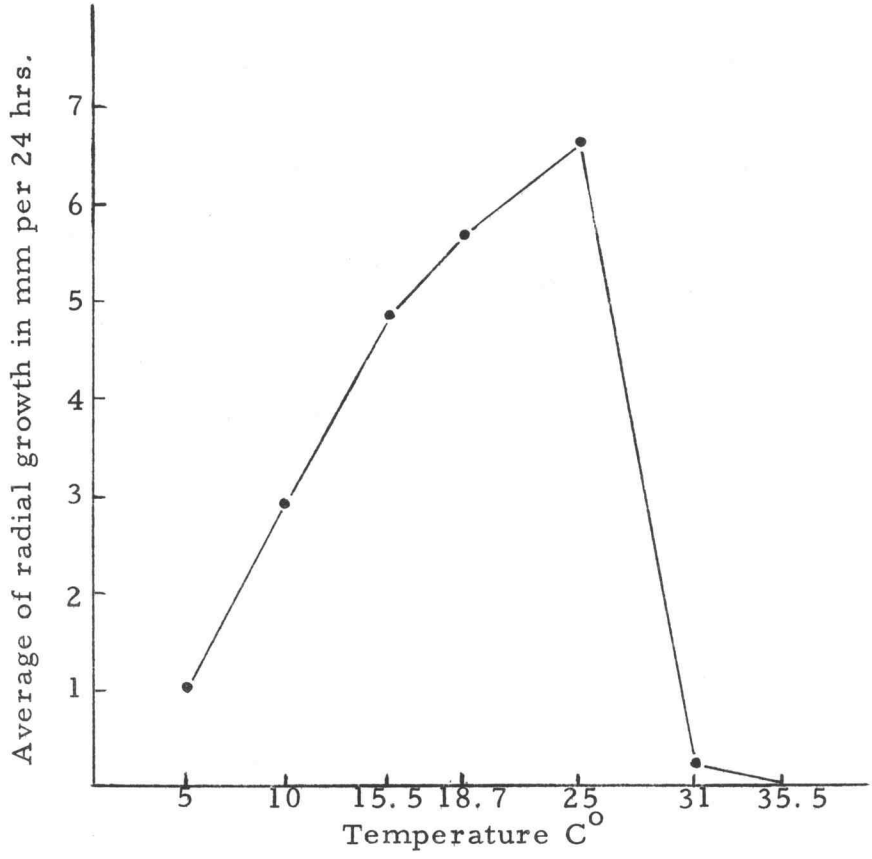

Figure 3. Effect of temperature on radial growth of Phoma menthae.

inoculation, except at 5° and 35.5°C where no growth was detected.

Growth of Phoma Menthae on Various Carbon Sources

The following carbon sources were tested: glucose, fructose, galactose, maltose, lactose, sucrose, and starch. The criterion of growth was the dry weight of mycelia and spores produced in liquid cultures. Harvests were made at 8, 14, and 18 days after inoculation. The pH of each carbon source at each harvest was measured and is presented in Table 3. The data on dry weights are presented in Table 2 and shown graphically in Figure 4. These data show that the decreasing order of carbon sources for growth of P. menthae as follows: starch, fructose, galactose, lactose, sucrose, maltose, and glucose.

Table 2. Growth of Phoma menthae on various carbon sources.

Carbon source	Dry weight in mg at indicated days after inoculation*			Dry weight (mg) per 24 hrs.
	8	14	18	
Glucose	38.8	68.0	83.1	4.6
Fructose	83.7	122.5	229.1	10.62
Maltose	39.8	59.0	86.7	4.63
Sucrose	43.1	68.5	92.6	5.13
Galactose	48.3	97.2	158.1	6.81
Lactose	46.8	77.3	98.0	5.62
Starch	166.0	196.2	237.2	15.9
No carbon	0.4	1.2	1.2	0.93

*Average of 4 replicates.

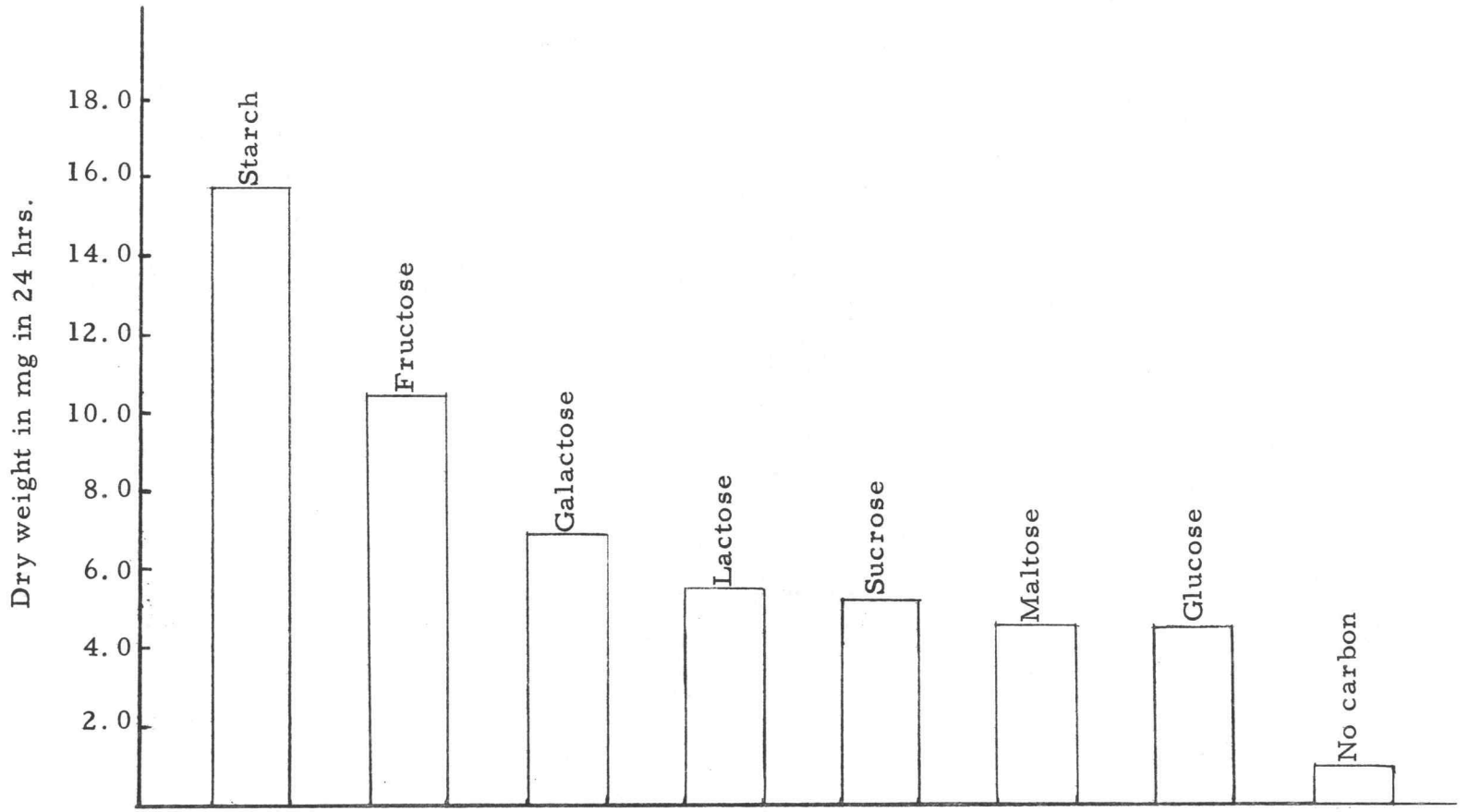

Figure 4. Growth of Phoma menthae on various carbon sources.

Table 3. Change of pH of various carbon source media during growth of Phoma menthae.

Media	pH of media*			
	Initial	1st harvest	2nd harvest	3rd harvest
Glucose	8.0	7.4	7.7	7.3
Fructose	7.8	7.5	7.9	8.0
Maltose	8.0	7.45	6.9	7.0
Sucrose	7.8	7.25	7.0	7.7
Galactose	8.3	7.5	7.6	7.8
Lactose	8.3	7.5	7.6	7.1
Starch	7.8	6.15	6.65	6.35
No carbon	7.8	7.75	7.8	7.85

*Average of 4 replicates.

The Disease

Methods of Inoculations

Wound and Non-Wound Inoculations. This experiment was performed to test the effect of three methods of inoculation on infection and disease severity. Inoculation methods were as follows: 1) a piece of culture media containing the fungus was placed in an incision wound on the plant stem, 2) the fungus was placed on a puncture wound, and 3) the fungus was placed on the stem without wounding. The effect on disease severity as measured by a rating scheme, is presented in Table 4 and shown

graphically in Figure 5. The disease developed most rapidly in plants which were inoculated in incision and stem puncture wounds. Disease development was slowest in plants inoculated without wounding.

Table 4. Effect of different methods of inoculation on disease severity caused by Phoma menthae in peppermint plants.

Inoculation Methods**	Disease rating* at indicated days after inoculation				
	7	10	13	16	21
A	2.8	3.35	3.4	3.5	3.65
B	1.6	2.2	2.3	2.45	2.65
C	2.5	3.05	3.4	3.55	3.55
D	0.0	0.0	0.0	0.0	0.0
E	0.0	0.0	0.0	0.0	0.0

*Disease rating;
Avg. of 20 plants:

0=No disease.
1=Light disease.
2=Moderate disease.
3=Severe disease.
4=Dead plants.

**Methods of inoculation:

A=Inoculation with incision wounding.
B=Inoculation without wounding.
C=Inoculation with stem puncture.
D=Non-inoculated wounded control.
E=Non-inoculated non-wounded control.

Harvest dry weights of the plants were determined 20 days after inoculation; the data are presented in Table 5 and shown graphically in Figure 6. Dry weights of the plants in all treatments were greatly reduced as compared with non-inoculated controls.

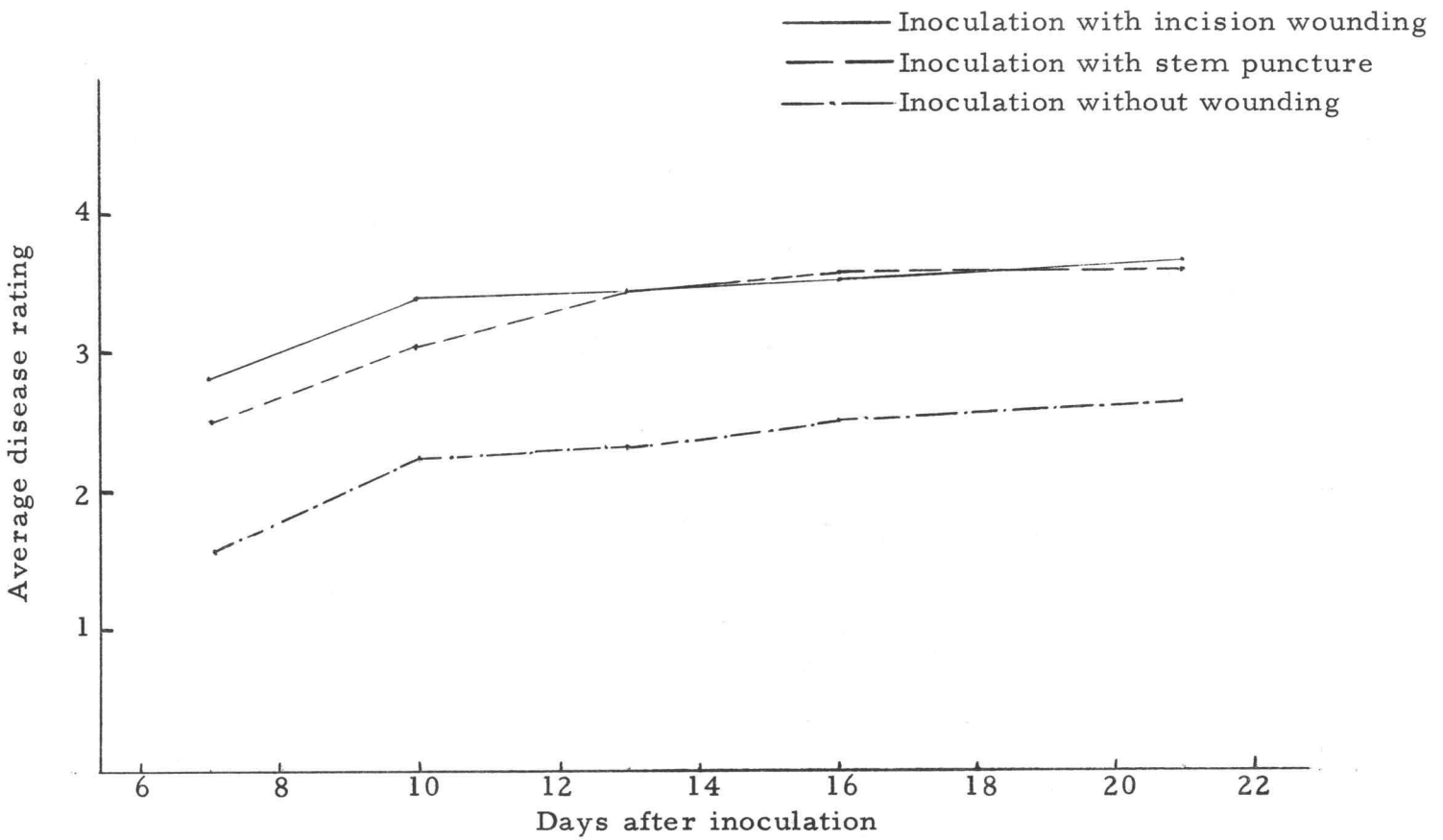

Figure 5. Effect of different inoculation methods on disease severity caused by Phoma menthae in peppermint plants.

Table 5. Effect of different methods of inoculation of Phoma menthae on dry weights of peppermint plants.

Plant No.	Dry weight (gm) 20 days after inoculation by different methods				
	A	B	C	D	E
1	0.90	--	0.61	0.76	0.67
2	0.52	0.18	0.18	0.75	1.85
3	0.41	0.51	0.79	1.61	1.33
4	0.14	0.68	0.61	1.47	0.71
5	0.25	1.50	0.19	0.86	1.92
6	0.47	0.51	0.15	0.85	1.41
7	0.31	0.94	0.43	0.87	1.53
8	0.28	0.16	--	1.27	1.19
9	0.37	0.54	0.30	0.44	2.36
10	--	2.15	0.50	1.76	1.16
11	0.24	--	0.25	2.74	3.53
12	0.63	--	0.70	1.20	0.98
13	--	0.68	0.24	1.93	0.46
14	0.34	0.18	0.28	0.49	--
15	0.23	1.37	0.13	1.08	--
16	0.38	0.63	0.29	1.35	--
17	--	0.66	0.51	1.23	--
18	1.05	--	1.01	0.52	--
19	0.12	0.54	0.57	0.90	--
20	0.27	--	1.01	2.03	--
Average	0.41	0.75	0.51	1.21	1.46

A=Inoculation with incision wounding.
B=Inoculation without wounding.
C=Inoculation with stem puncture.
D=Non-inoculated wounded control.
E=Non-inoculated non-wounded control.

Root Inoculation. This experiment was performed to test the effect of root inoculation on disease severity and harvest dry weights of plants. The roots were dipped in a spore suspension containing 100,000 spores per ml. The first symptoms that appeared on plants were red veins on the leaves, starting with the lower leaves first

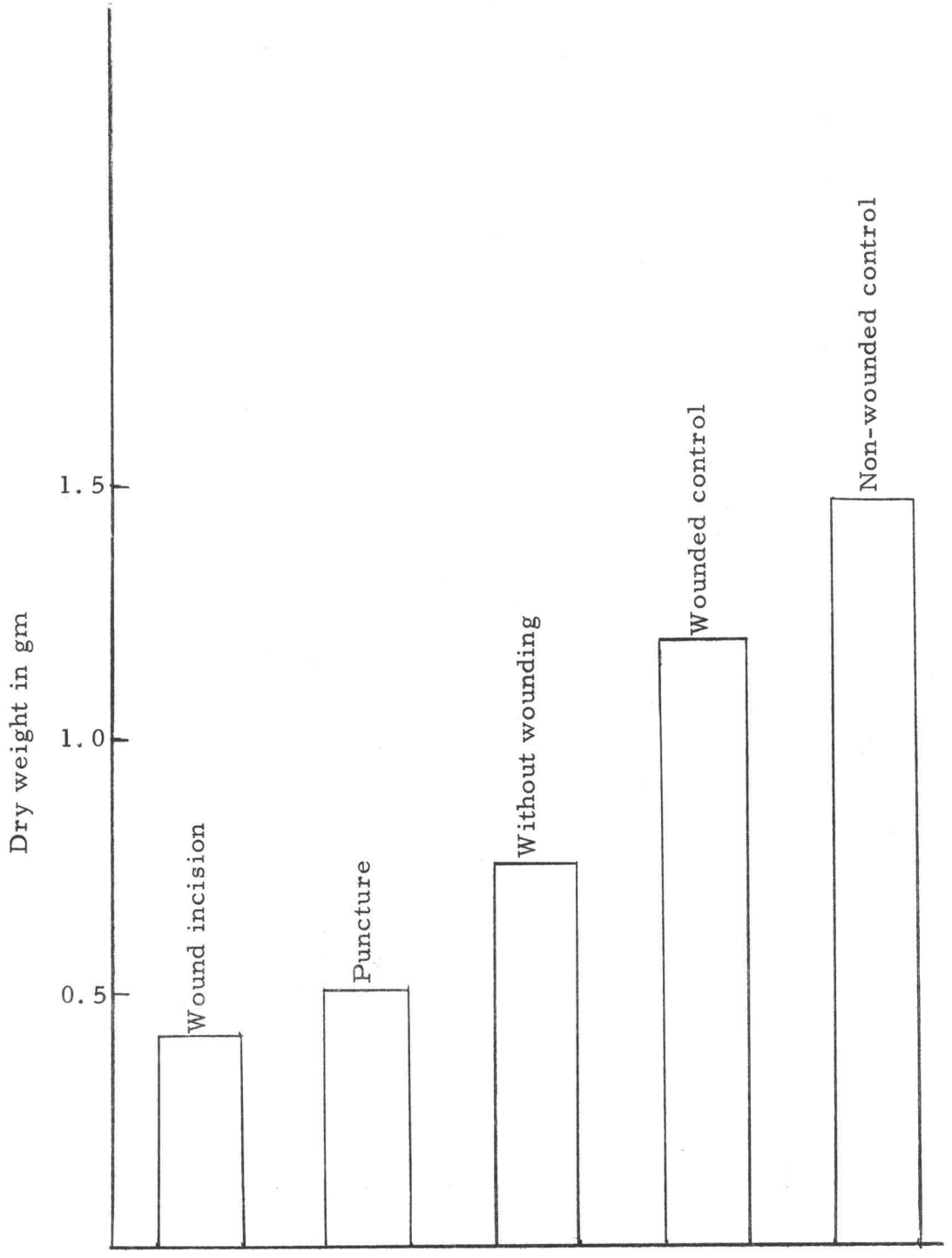

Figure 6. Effect of different inoculation methods on harvest dry weight of peppermint plants.

and then spreading to the entire plant. The plants became semi-wilted and then recovered. General stunting occurred as compared with non-inoculated controls; none of the plants died. Phoma menthae was isolated from the roots of stunted plants.

Harvest dry weights of the plants are presented in Table 6 and shown graphically in Figure 7. Harvest dry weights of infected plants were greatly reduced as compared with non-inoculated controls.

Table 6. Effect of root inoculation on harvest dry weight of peppermint plants.

Plant number	Dry weight in grams	
	Root inoculation	Control
1	1.17	2.44
2	1.66	3.15
3	1.33	2.39
4	1.21	5.10
5	0.86	3.60
6	0.62	3.46
7	1.04	3.23
8	1.30	4.78
9	1.15	3.36
10	1.41	4.74
Average	1.17	3.62

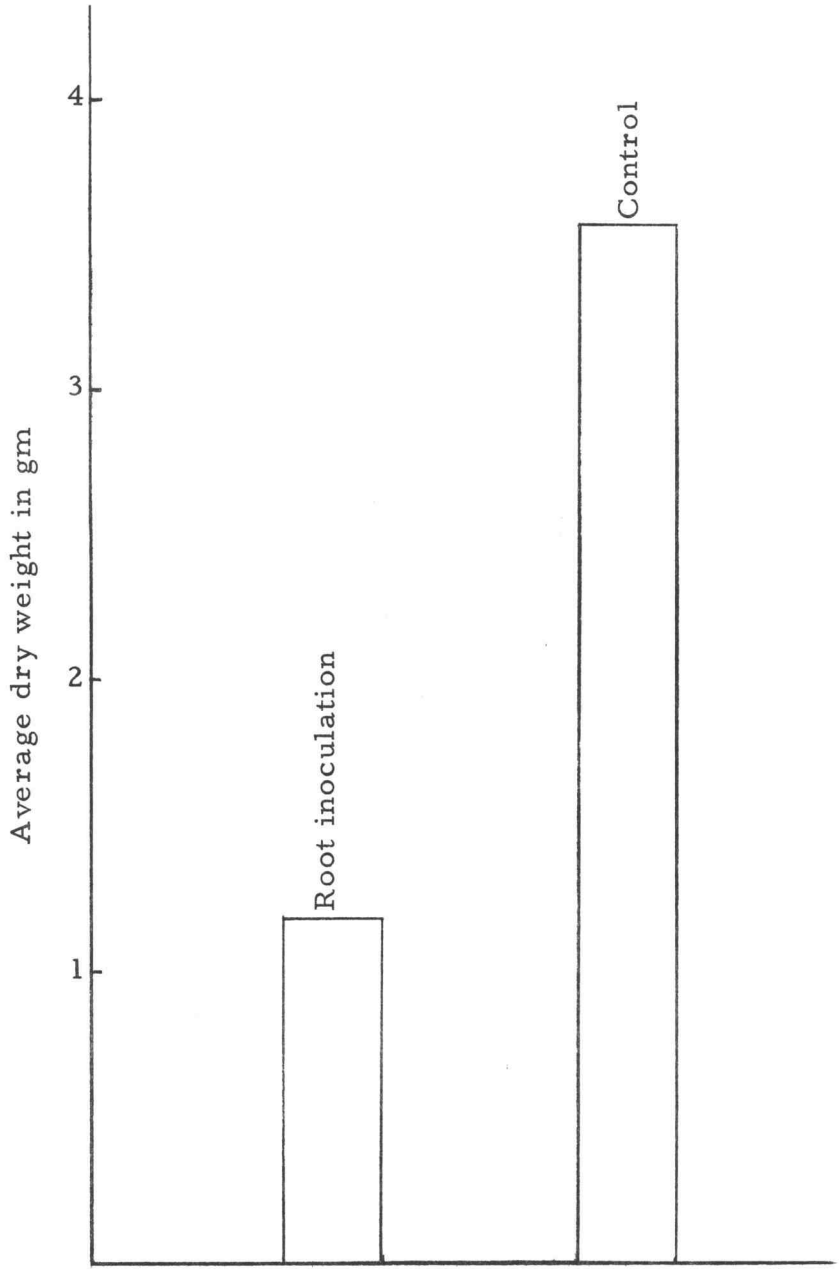

Figure 7. Effect of root inoculation on harvest dry weight of peppermint plants.

Effect of Temperature on Disease Developments

An experiment was performed to test the effect of temperature on development of cankers caused by Phoma menthae on mint rhizomes (Figure 1, Materials and Methods section). Temperatures tested were: 5°, 10.1°, 15°, 19°, 25.1°, and 30.1°C. The data are presented in Table 7 and shown graphically in Figure 8. The data show that the optimum temperature for development of cankers is between 19°-30.1°C. At all temperatures except 5°C, canker had developed after three days of incubation.

Table 7. Effect of temperature on canker development caused by Phoma menthae on rhizome tips of peppermint plants.

Temperature C°	Length of canker in mm at indicated days after inoculation*			Average increase in canker length (mm) per 24 hrs.
	3	4	5	
5.	-	-	-	-
10.1	1.86	4.6	8.6	1.23
15.	8.6	15.37	23.7	3.97
19.	12.25	19.8	27.7	4.97
25.1	14.75	21.7	29.2	5.47
30.1	2.0	2.6	3.6	0.68

*Average of 8 replicates.
-No canker developed.

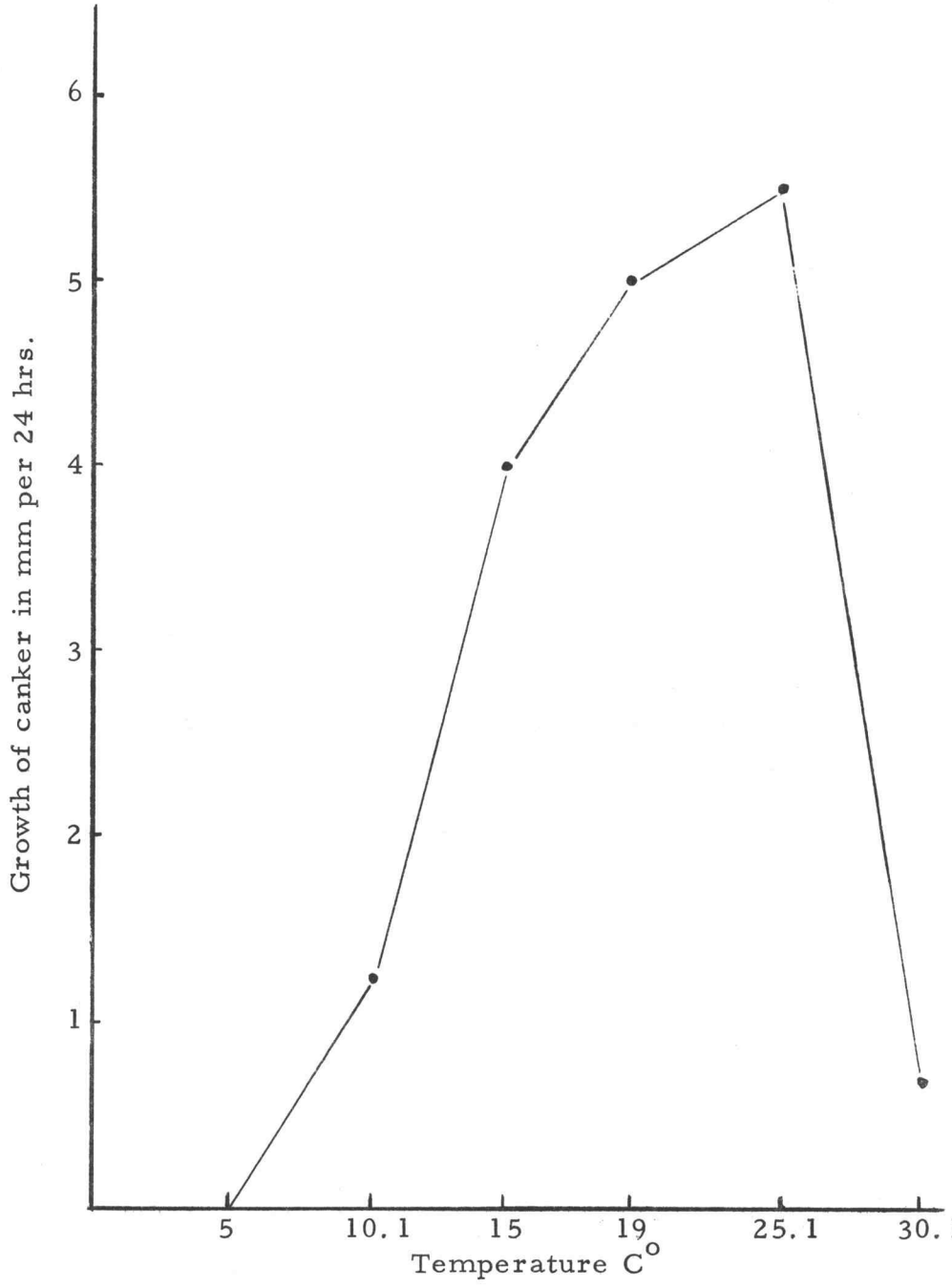

Figure 8. Effect of temperature on length of cankers caused by Phoma menthae on rhizome tips of peppermint plants.

Effect of Plant Age at Time of Inoculation on Disease Severity

Plants of different ages were inoculated by scratching the stem and applying fungal inoculum. Three groups of ten plants each were inoculated when 28, 34, and 41 days old. Another group of five plants was inoculated without scratching the stem when the plants were 41 days old; ten non-inoculated plants served as controls.

The data on disease severity are presented in Table 8 and shown graphically in Figure 9. Harvest dry weights of the plants is presented in Table 9 and shown graphically in Figure 10. Dry weights of plants inoculated when they were 28 days old was greatly reduced compared with plants in the other treatments, including the non-inoculated control.

Apparently a few plants escaped infection entirely, for example, plant number 1 at 28 days of age, number 3 at 34 days, and number 9 at 41 days (Table 9).

Table 8. Effect of plant age at time of inoculation on disease severity caused by Phoma menthae on peppermint plants.

Plant age (days)	Disease rating at indicated days after inoculation*														
	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
28	0.6	1.5	2.4	2.8		3.1	3.1				3.4	3.8		3.8	
34			0.7	1.8		2.4	2.7			3.4		3.4		3.4	
41					0.9	1.7	2.4	2.4		2.7		3.2		3.2	
41**								1.0	1.6		2		2.6		2.6
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

* Average reading of 10 plants, except treatment 41** is the average of 5 plants.

** Inoculated without wounding.

Table 9. Effect of plant age at time of inoculation on dry weight of peppermint plants.

Plant No.	Harvest dry weight (gm) of plants when inoculated at indicated ages				
	28 days	34 days	41 days	41* days	Control
1	6.53	7.20	0.69	4.60	5.75
2	0.37	0.38	0.80	1.22	4.80
3	0.58	9.27	1.06	1.47	9.91
4	0.20	0.41	0.90	8.12	7.43
5	0.23	0.48	0.89	0.44	7.27
6	0.14	0.57	1.18		5.91
7	0.16	0.53	5.95		6.71
8	0.25	0.51	0.67		9.25
9	0.35	0.70	9.54		8.07
10	0.15	0.65	0.86		8.24
Average	0.9	2.10	2.25	3.17	7.33

*The plants were inoculated without scratching the stem.

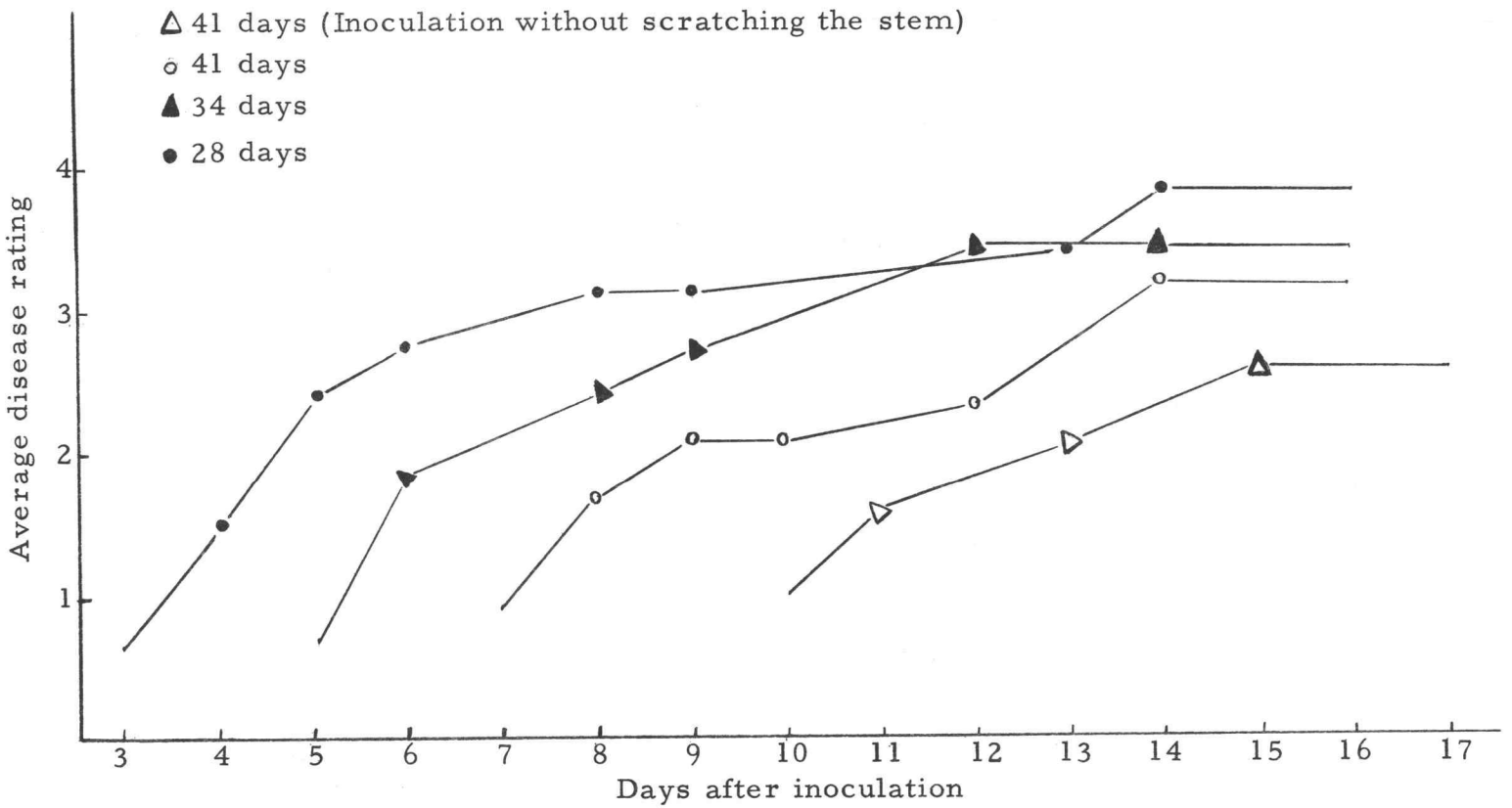

Figure 9. Effect of plant age at time of inoculation on disease severity.

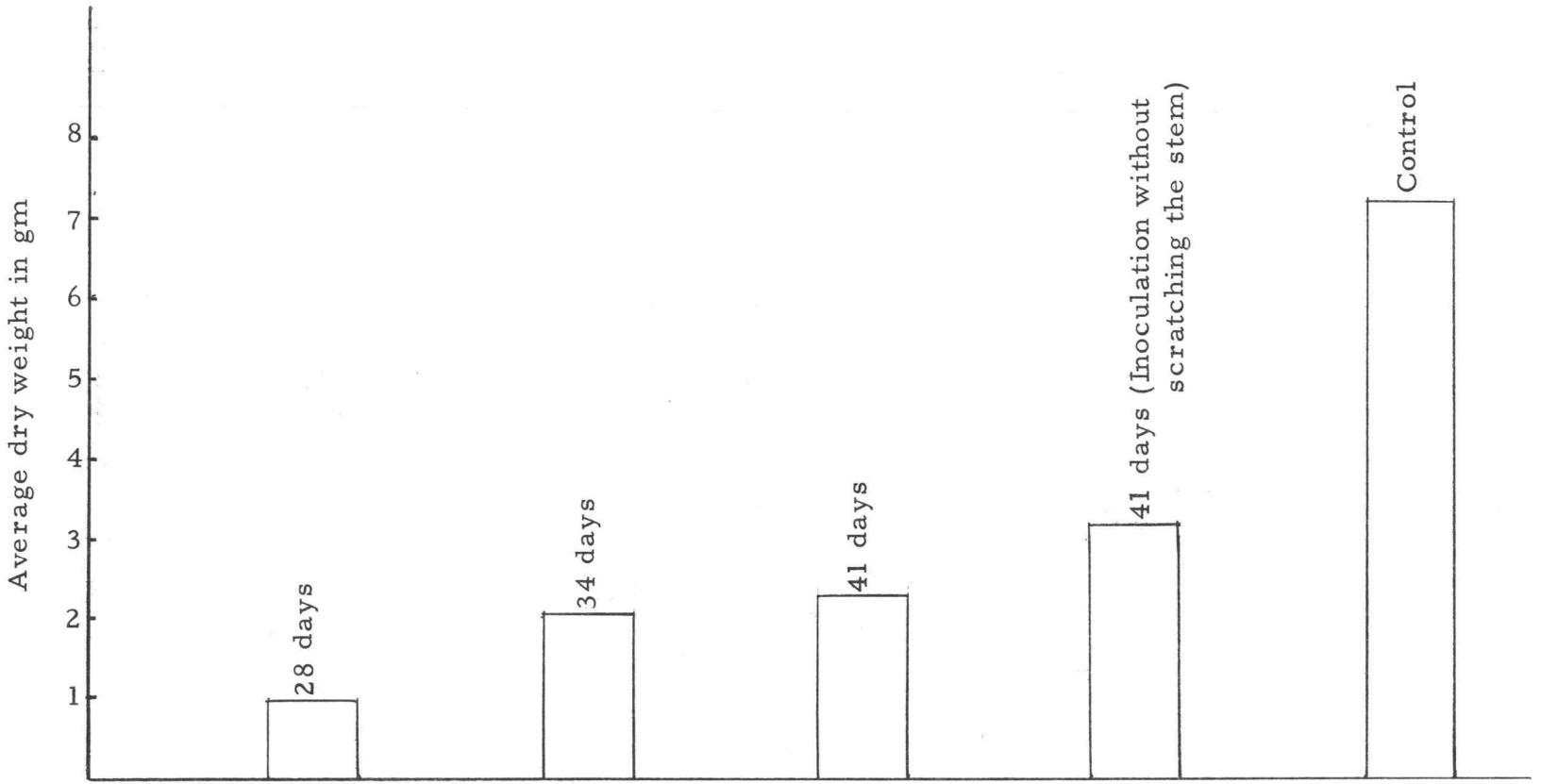

Figure 10. Effect of plant age at time of inoculation on harvest dry weight of peppermint plants.

DISCUSSION AND CONCLUSIONS

The fungus that causes black lesions and cankers on stems and rhizomes of Mentha piperita L. was tentatively identified as Phoma menthae Strasser. The causal agent was isolated from infected stems and rhizomes, grown in pure culture, and used to inoculate healthy plants where it caused symptoms identical to those originally observed. No previous description of a disease of Mentha spp. caused by Phoma was found in the literature.

Linear growth of P. menthae on Czapek Dox agar was greatest at 25°C. No growth occurred at 35.5°C, and little growth occurred at 5°C. These results compare favorably with those of Schenck and Gerdemann (13) and Rost (9) who worked with Phoma herbarum var. medicaginis and P. lini, respectively.

The optimum temperature for canker development in rhizomes of peppermint was between 19° and 30.1°C. The data on canker development at various temperatures showed a close relationship to data on growth made in culture at comparable temperatures.

Among seven carbon sources tested, growth of P. menthae in liquid culture was best with starch; growth with glucose, and maltose was surprisingly poor. The fungus grew well with fructose or galactose as carbon sources.

Inoculation of stems wounded by incision, puncture, or

scratches resulted in more rapid disease development than in comparable non-wounded stems. Infection occurred, however, in unwounded stems and moderate disease developed. These results agree with those of Bean and Wilcoxson (1) who obtained more lesions when P. herbarum var. medicaginis was used to inoculate alfalfa wounded with carborundum abrasive. Field observations have shown a high incidence of P. menthae infection of wounds on peppermint stems caused by mechanical cultivation.

Resistance of peppermint plants to the effects of P. menthae increases with age. Juvenile tissue is rapidly killed and young plants may die five to seven days after inoculation. Mature plants are less often killed by the disease even though stems may be extensively infected.

Root inoculation reduced growth of peppermint plants to about one-third that of healthy plants. This aspect of the host-parasite relationship needs further study.

The very rapid development of stem cankers followed by complete collapse of tissue when young plants were inoculated suggests that P. menthae possesses very active pectolytic or cellulolytic enzymes. This aspect of the disease also needs further study.

SUMMARY

Phoma menthae Strasser causes black lesions and cankers on stems and rhizomes of Mentha piperita L. The fungus was isolated from infected rhizomes and stems.

The optimum temperature for growth of the fungus in culture was between 20-25°C; little growth occurred at 5°C.

Among seven carbon sources tested in liquid culture, Phoma menthae grew best on starch and poorest on glucose and maltose. The fungus grew well with fructose or galactose as carbon sources.

Inoculation of healthy peppermint stems with or without wounds, produced typical symptoms of the disease. Disease developed most rapidly, however, in plants inoculated in wounds. Dry harvest weights of plants inoculated in wounds were greatly reduced as compared with those inoculated without wounds. Phoma menthae was reisolated from infected plants.

Root inoculation reduced greatly the dry harvest weights of inoculated plants as compared with controls. The fungus was re-isolated from roots of stunted plants.

The disease developed rapidly on peppermint rhizomes between 19-25°C; no disease developed at 30. 1C.

Plants inoculated at different ages demonstrated that resistance to the action of the fungus increased with plant age.

BIBLIOGRAPHY

1. Bean, G. A. and R. D. Wilcoxson. Development of spring black stem on alfalfa and red clover. Crop Science 1(4): 233-235. 1961.

2. Björling, K. Undersökningar rörande Phoma betae (Oud.) Fr. med särskild hänsyn till en av svampen orsakad stjälkröta på Betfröplantor. [Investigations relating to Phoma betae (Oud.) Fr. with special reference to a stem rot of beet seed plants caused by the fungus]. (Abstract) The Review of Applied Mycology 25:54-55. 1946.

3. Ch'iao, Y. P. Fomoz Morkovi v usloviyakh Moskovskai oblasti [Phomopsis of carrot in the Moscow region.]. (Abstract) The Review of Applied Mycology 41:422. 1962.

4. Gerstner, W. Untersuchungen über den Sellerieschorf. [Studies on celery scab.]. (Abstract) The Review of Applied Mycology 32:230. 1953.

5. Gerstner, W. Untersuchungen über die Biologie des Erregers des Sellerieschorfes (Phoma apiicola Klebahn). [Studies on the biology of the agent of celery scab (Phoma apiicola Klebahn).]. (Abstract) The Review of Applied Mycology 32:604. 1953.

6. Heiberg, Barbara C. and G. B. Ramsey. Phoma rot of garden beets. Phytopathology 38(5):343-347. 1948.

7. Kemp, W. G. A new root rot of florists Chrysanthemums in Ontario. Canadian Journal of Plant Science 38(4):464-476. 1958.

8. Renfro, B. L. Studies concerning epidemiology of black stem on alfalfa. (Abstract) The Review of Applied Mycology 41:43. 1962.

9. Rost, H. Untersuchungen über einige krankheiten des Leins in Deutschland. [Investigations on some flax diseases in Germany.] (Abstract) The Review of Applied Mycology 18:315-316. 1939.

10. Rüdiger, W. Untersuchungen über die Pustelkrankheit des Leins (Phoma lini). [Research on the pustule disease of flax (Phoma lini).]. (Abstract) The Review of Applied Mycology 30:107. 1951.

11. Rüdiger, W. Zur pathologischen Anatomie des Leins, Linum usitatissimum L. [On the pathological anatomy of flax, Linum usitatissimum L.]. (Abstract) The Review of Applied Mycology 32:187. 1953.

12. Saccardo, P. A. Sylloge fungorum omnium hucusque cognitorum. Vol. 22. Patarii, by the author, 1913. 1506 p. (Supplementum universale, pt. 9)

13. Schenck, N. C. and J. W. Gerdemman. Taxonomy, pathogenicity, and host parasite relations of Phoma trifolii and Phoma herbarum var. medicaginis. Phytopathology 46(4): 194-200. 1956.

14. Schneider, Roswitha. Uber einen bemerkeswerten Befall an kohlrabi, verursacht durch Phoma lingam (Tode) Desm. [On a noteworthy disease of kohlrabi caused by P. lingam.]. (Abstract) The Review of Applied Mycology 40:572. 1961.

15. Van Hoof, H. A. Vallers en Kanker in Bewaarkool. [Dry rot and canker in stored cabbage.]. (Abstract) The Review of Applied Mycology 39:358. 1960.

16. Walker, J. C. Plant pathology. New York, McGraw-Hill, 1957. 707 p.

17. Young, P. A. Tabulation of the genera Phoma and Macrophoma. 43 p. (Undated manuscript received August 1962 from Dr. G. A. Gries, University of Arizona, Tucson).