

DISEASE CYCLE AND CONTROL OF PEPPERMINT  
RUST CAUSED BY PUCCINIA MENTHAE PERS.

by

CHESTER ELLSWORTH HORNER

A THESIS

submitted to

OREGON STATE COLLEGE

in partial fulfillment of  
the requirements for the  
degree of

DOCTOR OF PHILOSOPHY

June 1954

APPROVED:

Redacted for Privacy

---

Professor of Botany and Chairman of Department of  
Botany

In Charge of Major

Redacted for Privacy

---

Chairman of School Graduate Committee

Redacted for Privacy

---

Dean of Graduate School

Date thesis is presented May 5, 1954

Typed by Ruth Brown

### ACKNOWLEDGEMENT

The author wishes to express his appreciation to Dr. S. M. Dietz for suggesting the problem and for counsel during the course of research and preparation of the manuscript.

Messrs. Earl Chartrey, Roy Engbretson, Cecil Manning, Jack Sather and Joe Wayman generously contributed their time and equipment during much of the field work for which the author is grateful.

Acknowledgement is made to the Beech-Nut Packing Company, I. P. Callison and Sons, A. M. Todd Company and Wm. Wrigley Jr. Company for assistance on oil flavor evaluations.

The financial assistance provided by the Beech-Nut Packing Company is gratefully acknowledged.

# TABLE OF CONTENTS

	Page
INTRODUCTION	
REVIEW OF LITERATURE. . . . .	3
The Crop . . . . .	3
The Fungus . . . . .	3
The Disease. . . . .	4
Control. . . . .	6
GENERAL METHODS . . . . .	8
Laboratory and Greenhouse Methods. . . . .	8
Spore germination . . . . .	8
Pathological histology. . . . .	9
Inoculation . . . . .	9
Greenhouse cultural methods . . . . .	10
Field Methods. . . . .	10
DISEASE CYCLE AND FACTORS AFFECTING IT. . . . .	12
Method of Overwintering. . . . .	12
Infection by Basidiospores . . . . .	15
Aeciospore Cycle . . . . .	20
Urediospore Cycle. . . . .	28
CROP LOSSES CAUSED BY PEPPERMINT RUST . . . . .	42
Losses Caused by Basidiospore Infection, Spermogonia and Aecia . . . . .	42
Losses Caused by the Urediospore Stage . . . . .	45
Losses Caused by the Teliospore Stage. . . . .	52
METHODS OF EVALUATING RUST SEVERITY AND CONTROL	
DATA . . . . .	53
Evaluation of Rust Severity. . . . .	53
Evaluation of Control. . . . .	62
CONTROL OF PEPPERMINT RUST. . . . .	63
Cultural Practices . . . . .	63
Eradication of Wild and Escaped Mint . . . . .	68
Eradication of the Aecial Stage by Chemicals . . . . .	72
Protectant Fungicides. . . . .	77
Fungicide screening trials. . . . .	77
Flavor evaluation of peppermint oil from plots treated with fungicides. . . . .	82
Fungicidal sprays and dusts . . . . .	83
Resistant Varieties. . . . .	87
SUMMARY AND CONCLUSIONS . . . . .	94
BIBLIOGRAPHY. . . . .	98



# TABLES

	Page
1. Germination of teliospores collected from the field at different dates. . . . .	15
2. Time of basidiospore infection and spermogonia and aecia formation on young mint shoots under field conditions in Oregon in 1952-'53. . . . .	19
3. Influence of temperature on the development of spermogonia and aecia of mint rust. . . . .	21
4. Comparison of the length of the incubation period necessary for the production of aeciospores under field and greenhouse conditions during February, March and April . . . . .	24
5. Effect of temperature and storage time on aeciospore viability. . . . .	26
6. Viability of urediospores taken at different dates from mint foliage exposed to field conditions. . . . .	30
7. Influence of relative humidity on infection by mint rust urediospores . . . . .	34
8. Comparison of the percentage of viable urediospores collected from sori exposed to and protected from direct solar radiation. . . . .	37
9. Effect of temperature, length of exposure and incubation time on death of rust mycelium and infected leaf cells. . . . .	40
10. The percentage of peppermint shoots killed in four different fields by invasion of the aecial lesions by secondary organisms . . . . .	44
11. Comparison of leaf drop from heavily and lightly rust infected peppermint plants . . . . .	46
12. Comparison of oil yield from heavily and lightly rust infected peppermint. . . . .	47
13. Comparison of oil yield from heavily and lightly rust infected peppermint from 3 different fields. . . . .	48

# TABLES (continued)

	Page
14. Comparison of number, size and weight of rhizomes produced by heavily rust infected and rust free peppermint plants. . .	51
15. Number of oil glands per unit area on peppermint leaves with four classes of rust infection and from three positions on the plant. . . . .	58
16. Yield of oil from peppermint leaves with 4 degrees of rust infection collected from 3 positions on the plants. . . . .	61
17. Statistical analysis of the correlation of disease index and yield. . . . .	62
18. Effect of different plowing practices on the incidence of the aecial stage of mint rust . . . . .	65
19. Effect of cultivation on the survival of aecial infected peppermint shoots . . . . .	66
20. Effect of irrigation on rust severity and leaf drop . . . . .	68
21. Relative effectiveness of different herbicides for killing rust infected mint plants before aeciospore discharge . . . . .	71
22. Effectiveness of 8 treatments for the eradication of the aecial stage of mint rust . . . . .	74
23. Comparison of the effectiveness of 2 chemical treatments for the eradication of the aecial stage of mint rust and their effect on subsequent rust control . .	75
24. Effect of chemical burn back on the number of mint shoots per square yard six weeks after treatment . . . . .	76
25. Effectiveness of 10 materials for rust control as shown by a computed disease index . . . . .	79-80

# TABLES (continued)

	Page
26. Effectiveness of 10 materials for rust control as shown by yield of oil. . . . .	81
27. Comparison of disease index and oil yield means from peppermint plots treated with 10 fungicides. . . . .	82
28. Effectiveness of Phygon sprays and dusts at different rates and times of application for mint rust control as shown by a computed disease index. . . . .	88
29. Resistance of 5 species and 91 hybrids of <u>Mentha</u> to race 2 of <u>Puccinia menthae</u> . . . .	91
30. Rust reaction produced on 2 species and 5 hybrids of <u>Mentha</u> by aeciospores collected from 3 mint species in 12 localities. . . .	93

## FIGURES

	Page
1. Mixed uredial and telial sori on peppermint stems collected from the field. . . . .	13
2. Cross sections of a peppermint stem with spermogonia and aecia (A) and an aecium (B). . . . .	17
3. Mature aecia on young peppermint shoots. Some shoots with multiple infections. Note hypertrophy, twisting and distortion .	23
4. Rate and direction of spread of mint rust from a single aeciospore source. Each concentric circle represents 4 feet . . . .	29
5. Comparison of relative humidity in the open and under dense mint foliage at different heights above-ground and at different hours of the day. . . . .	33
6. Death of rust infected tissue caused by high temperature. Leaves collected from the field (A); and leaves given 100° F. treatment for 4 hours 8 days after inoculation (B) . . . . .	38
7. Comparison of leaf drop and lateral stem development on healthy (left) and severely rust infected (right) peppermint plants . .	49
8. Destruction of oil glands on leaf surfaces by different degrees of rust. A, none; B, light; C, moderate; D, severe rust infection . . . . .	55
9. Rust rating standard used to evaluate rust severity and control data. Rust classes 0, 1, 2 and 3 represent 0, 0-5, 6-25 and 26-50 per cent reduction in oil gland numbers respectively. . . . .	60
10. Duster used in fungicidal control trials. . . .	85
11. Sprayer used in fungicidal control trials . . .	86

DISEASE CYCLE AND CONTROL OF PEPPERMINT  
RUST CAUSED BY PUCCINIA MENTHAE PERS.

INTRODUCTION

Culture of peppermint (Mentha piperita L. var. Mitcham) for its essential oil has been carried on in Oregon since about 1909. The industry grew slowly at first, then expanded rapidly since 1940. Peppermint is grown in Oregon chiefly on the rich, river bottom soils of the Willamette River and its main tributaries. It is grown to a lesser extent on peat and mineral soils along the lower Columbia River.

Mint rust (Puccinia menthae Pers.) was first found in Oregon in commercial mint plantings of the lower Columbia River area in 1948. In the summer of 1949 economic losses occurred in certain fields in Columbia county and by November every mint field was infested with rust (26). Mint rust appeared in Willamette Valley mint fields in the spring and summer of 1950 and by November every mint field examined in Western Oregon was infested (26).

Rust on common spearmint (Mentha spicata L.) and Scotch spearmint (M. cardiaca L.) has been a seasonally important disease in the Midwest for many years (23, p. 17), but rust was not reported on peppermint in that area until 1952 (17). The races of the mint rust fungus attacking common spearmint and peppermint are distinctly different



in pathogenicity and not cross-infective on these two mint species (4). Mint rust has been known on both spearmint and peppermint in Europe and parts of Russia for many years (6) and (27). Control measures for mint rust developed by European and Russian investigators are not generally applicable in the United States because of wide differences in cultural practices.

The disease cycle of peppermint rust in the field has not previously been investigated in this country. When mint rust became epiphytotic in the Northwest in 1949, research on the disease cycle and control of this disease was undertaken and is reported in this thesis.



## REVIEW OF LITERATURE

## The Crop

According to Nelson (16, p. 30) the peppermint industry began in Oregon in 1909 with planting stock from Michigan. Nelson (16, pp. 1-30) ascribes the origin of the present commercial variety of peppermint (Mentha piperita L. var. Mitcham) to the natural hybrids (M. sylvestris x M. rotundifolia) x M. aquatica and traces its culture from England to the United States.

Commercial peppermint is male sterile (16, pp. 192-196) and is propagated mainly by rhizomes which are produced abundantly. Peppermint production in Oregon has increased rapidly since 1940. The average total acreage in Oregon from 1936 to 1945 was 3,620 acres (23, p. 3). In 1946 acreage was increased to 9,000 (23, p. 3) and by 1952 had reached 15,000 acres.\*

## The Fungus

The mint rust fungus, (Puccinia menthae), first described by Persoon in 1801 (21, p. 227) on Mentha aquatica, has been recorded on many genera and species of the family Labiatae from all over the world. In 1934 Arthur (2, pp.

\*Records from a private source.

327-329) recorded mint rust on 48 species and 9 genera of the family Labiatae in the United States and Canada.

Puccinia menthae is an autoecious full-cycle rust belonging to the family Pucciniaceae in the order Uredinales. It is composed of numerous pathogenic races which can be differentiated by their reaction on suitable genera, species and varieties of the Labiatae. In 1906 Cruchet (6) differentiated 8 pathogenic races in Europe. Recently Baxter and Cummins (4) have demonstrated the presence of 15 races in North America.

#### The Disease

Vergovsky (27) studied the life cycle of mint rust in the Crimea. He demonstrated that rust mycelium was not systemic in the peppermint plant, and that overwintering of teliospores was necessary for new infections to be initiated the following spring. Rust caused losses of 20 to 50 per cent of the yield of oil depending on meteorological conditions, and further, the menthol content of oil from rust infected leaves was less than from healthy leaves. He listed the time lapse from basidiospore infection to aeciospore production on peppermint as 10-12 days; from aeciospore infection to urediospore production 12-15 days; and from urediospore infection to urediospore production 18-20 days. Only young, tender stem or leaf

tissues could be infected by basidiospores, mature plant tissues were not infected.

Mint rust has caused serious losses on peppermint, Mentha piperita L. (14), 26) and 27)); spearmint, M. spicata L. (23, p. 17), and M. cardiaca L. var Scotch (4); and on Japanese field mint M. arvensis L. var piperascens (9). These mints are grown as field crops for their essential oil which is removed by steam distillation of the hay. Spearmint is also grown in the winter as a forced greenhouse crop near many large cities where the leafy shoots are sold as a flavoring for foods and drinks. In 1945 Niederhauser (18) published the results of a comprehensive study of the rust disease of greenhouse-grown spearmint and its control. His work on the life cycle of mint rust agreed with that done earlier by Vergovsky (27). Hot water treatment of rhizomes before planting them in the greenhouse was an effective method of control.

Muhle (14) in Germany has recently substantiated the earlier work of Vergovsky on the disease cycle of the peppermint rust fungus.

Rust on commercially grown peppermint was first reported in the United States by Steenland (26) in Oregon where it became epiphytotic in 1949. Later Baxter (3) reported its occurrence in Western Ontario, Canada in 1951

and Nelson (17) in Michigan in 1952.

### Control

Vergovsky (27) demonstrated that plowing the mint stubble under in the fall to a depth of at least 5 cm. was effective in greatly reducing the amount of spring infection. Sulfuric acid used to kill the regrowth in the fall also greatly reduced spring infections by preventing the production of overwintering teliospores on the foliage. Copper sulfate and Bordeaux sprays were not effective in protecting the foliage from severe infection. Sulfur, while more effective against rust reduced the accumulation of oil in the plants. Vergovsky recommended early cutting as a means of avoiding excessive leaf drop due to heavy rust infection. Dusting with sulfur or spraying with Bordeaux has been recommended for rust control on spearmint in the Midwest (23, p. 17) but is not generally practiced.

Muhle (14) has suggested that some control can be obtained by such cultural practices as avoiding heavy nitrogen fertilization, harvesting before rust is severe and crop rotation. Cuttings taken in the fall, submerged in formalin, copper sulfate, Germisan, Ceresan and Aretan solutions of 0.4 to 2 per cent for varying lengths of time

were rust free the following spring. Injury of the cuttings was noted at the higher concentrations and longer durations.



## GENERAL METHODS

Certain general laboratory and field methods were followed throughout the course of this investigation and will be described in this section. Special methods were applied to individual experiments and will be described in later sections.

### Laboratory and Greenhouse Methods

#### Spore germination

Teliospore germination tests were made frequently during the course of studies on overwintering, time of germination and infection, and length of viability in the field. Telia bearing leaves were collected, the spores removed with a scalpel and floated on 2 per cent sucrose solution. For maximum germination teliospores floating on 2 per cent sucrose solution in Petri plates were held at 15° C. in controlled temperature cabinets.

Aeciospore and urediospore germination tests were made in conjunction with experiments on overwintering, effects of temperature, length of viability and inhibition of germination by chemicals. Spore suspensions were made by washing leaves or shoots with sporulating sori in flasks of distilled water. Drops of the spore suspension



were pipetted onto solidified 1.7 per cent water agar in Petri plates. For maximum germination the agar plates were held at 20° C. for 24 to 48 hours.

### Pathological histology

Rust infected tissues were studied by histological methods. Sections of diseased leaves and stems were killed in F.A.A. and dehydrated with tertiary butyl alcohol. The tissues were then infiltrated and embedded with paraffin and sectioned on a rotary microtome. The most satisfactory stain used was iron hematoxylin and eosin.

### Inoculation

Inoculations of disease free plants with teliospores, aeciospores and urediospores were made frequently during the course of this investigation. Spores were suspended in water and sprayed on leaves, stems and buds with a DeVilbiss No. 15 atomizer. When uniform spore suspensions were used the number of spores per ml. was determined with a Neubauer haemocytometer and the spore suspension adjusted to the desired level by the addition of water. After inoculation plants were placed in moist chambers for 24 to 48 hours, then removed to the greenhouse bench.

Inoculation of young shoots and buds with teliospores

consisted of placing teliospores on the leaves of buds or on the young shoots with a camel's hair brush. The inoculated plants were then placed under bell jars or plastic covers for 48 hours.

#### Greenhouse cultural methods

Several phases of this investigation were carried out in the greenhouse under uniform methods of soil preparation and culture. Soil consisted of a mixture of sandy loam, sand and peat moss to which a 16-20-0 commercial fertilizer was added. All soil was thoroughly mixed and screened.

Test plants were grown from rhizomes and cuttings. When test plants of uniform size were desired these were selected from cuttings rooted for 2 to 3 weeks in sand.

During the period from October to March supplementary light was used to simulate a 14 to 18 hour day length.

#### Field Methods

The experimental design used in most field tests was a randomized block. The number of replications was varied to meet the needs of the individual experiments.

Mass inoculation of field plots was made by spraying a suspension of urediospores in water onto the foliage

from a 3 gallon Hudson knapsack sprayer. A variable direction nozzle was used to direct the spray on the undersides of the leaves. Following inoculation the plots were watered with an overhead sprinkler irrigation system for 12 hours.

## FIELD DISEASE CYCLE AND FACTORS AFFECTING IT

### Method of Overwintering

The mint rust fungus overwinters as teliospores on fallen leaves, mint stubble, rhizomes and the soil surface. Vergovsky (27) established that the fungus did not overwinter as perennial mycelium. This has been substantiated by Niederhauser (18) and Muhle (14).

Teliospores are produced from May to December on mint plants in the field under Oregon conditions. They have been observed occasionally in May, June, July and August on mint plants which were subject to unfavorable environmental conditions. Low temperature, low light intensity and nutrient deficiency are factors closely associated with teliospore production during the spring and summer months. Teliospores have not been observed before July 25 on plants growing under favorable environmental conditions.

Teliospores may be produced on leaves, stems and rhizomes. Figure 1 shows mixed telial and uredial sori on mint stems collected from the field August 27, 1952.

The greatest source of overwintering teliospores is on the regrowth which becomes heavily infected after harvest. In Oregon the shift from production of urediospores



Figure 1. Mixed uredial and telial sori on peppermint stems collected from the field.

to teliospores takes place during October.

Teliospores of Puccinia menthae require a short period of dormancy before they are capable of germination. Niederhauser (18, p. 14) found a minimum dormancy period in the greenhouse of 12 days after the telium broke through the epidermis until the spores germinated. The dormancy period in the field cannot be assumed to be the same because of greater extremes of moisture and temperature which are known to affect dormancy of teliospores of other rust species (1, pp. 209-225).

During the middle of October, 1951, conditions were favorable for rust infection. Urediospore infections that took place during that time produced almost 100 per cent telial sori which began to sporulate by November 1. To determine the time of maximum germination of teliospores that were subject to field conditions, several mint plants were examined November 10 and selected leaves on the mint regrowth bearing newly erupted telial sori were marked. At approximately 20 day intervals collections of teliospores were made from 5 of the marked leaves and brought into the laboratory for germination tests. The spores were floated on 2 per cent sucrose solution in Petri plates and the plates incubated for 48 hours at 15° C. Each plate contained spores from one leaf and 50 spores were counted in



each plate and the number germinated recorded (Table 1).

Table 1. Germination of teliospores collected from the field at different dates.

Date collected	Number of spores in 50 germinated from leaf number					Total no. germinated	Per cent germination
	1	2	3	4	5		
Nov. 10, 1951	8	11	6	9	7	41	16.4
Nov. 19, 1951	11	14	9	17	15	66	26.4
Dec. 7, 1951	17	14	21	19	23	94	37.6
Dec. 23, 1951	19	20	17	29	31	116	46.4
Jan. 13, 1952	33	24	21	33	30	141	56.4
Jan. 24, 1952	19	11	14	9	10	63	25.2
Feb. 15, 1952	11	14	16	9	11	52	20.8
Feb. 25, 1952	9	6	11	4	8	38	15.4

Teliospore germination occurred over the entire period of 108 days included in this test and maximum germination occurred with spores collected in December and January.

#### Infection by Basidiospores

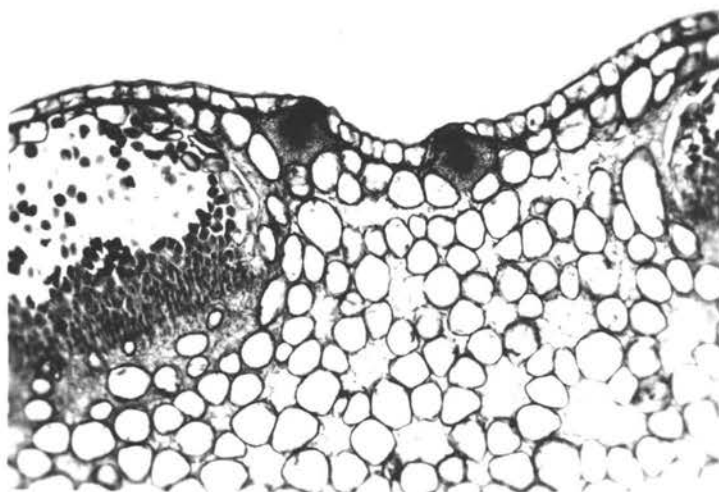
Niederhauser (18, pp. 14-21) described the phenomena associated with basidiospore infection and subsequent

development of spermogonia in spearmint rust. Vergovsky (27) found the period of time necessary from basidiospore infection to production of aeciospores was 10-12 days under laboratory conditions. Figure 2 shows cross sections of a peppermint stem with spermogonia and aecia.

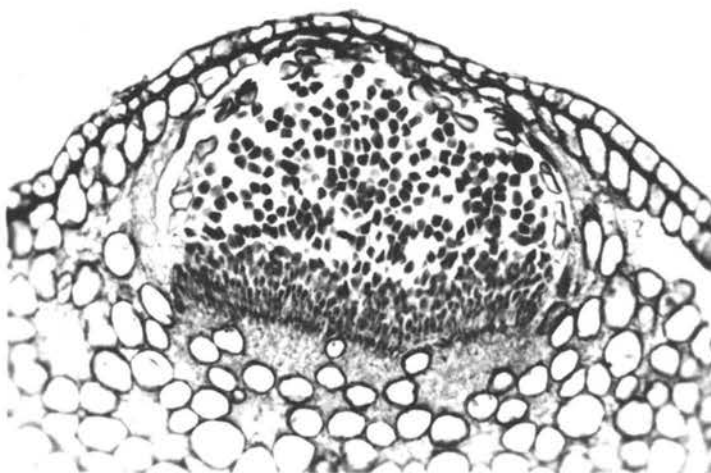
Field observation in Oregon suggested that infection by basidiospores takes place in December and January, but mature aecia have not been recorded before February 9. A test was made to determine when basidiospore infection takes place in the field and the time lapse between basidiospore infection and aeciospore production under field conditions.

An unplowed field with heavy teliospore production on the regrowth and rhizomes was selected in November 1952. Beginning December 1, 25 young shoots from 1 to 2 inches long were collected at approximately 2-week intervals until March, 1953, when the field was plowed. The young shoots were placed in plastic bags containing moist paper towels and brought to the laboratory.

The shoots were washed, trimmed of roots, placed between rectangular pieces of potato tuber and sectioned at 30 microns on a sliding microtome. The sections were floated in water, placed in a water suction apparatus to evacuate air bubbles in the intercellular spaces, stained



A



B

Figure 2. Cross sections of a peppermint stem with spermatophytes and aecia (A) and an aecium (B).

with 0.5 per cent methylene blue in lactophenol, mounted on microscope slides and examined under a microscope for evidence of infection, spermogonia, and aecia formation.

Visible external symptoms of infection were first observed on shoots collected December 30, however, internal intercellular rust mycelium and characteristic intracellular haustoria were observed microscopically in sections from shoots collected December 13 (Table 2).

Spermogonia formation was first observed December 30, but aeciospores were not produced until February 17, 65 days later. Thus, under field conditions from December to February a much longer time is required for basidiospore infections to ultimately result in aeciospore production than 10 to 12 days as reported by Vergovsky (27) for laboratory conditions.

The first external symptoms of infection on stems, petioles and leaf veins consist of pink or red, slightly raised blisters 1 to 3 millimeters in diameter. These blisters gradually enlarge and may attain a length of 3 centimeters on large, vigorous shoots. It is possible to recognize infections caused by basidiospores very early in their development by the small red blisters they cause, therefore, the effect of temperature on the development of spermogonia and aecia was tested.

Table 2. Time of basidiospore infection and spermogonia and aecia formation on young mint shoots under field condition in Oregon in 1952-'53.

Date shoots collected	Number of 25 shoots showing:			
	Basidiospore infection	Spermogonia formation	Aecia formation	Aecia sporulation
Dec. 1, 1952	0	0	0	0
Dec. 13, 1952	3	0	0	0
Dec. 30, 1952	11	3	0	0
Jan. 11, 1953	10	4	2	0
Jan. 15, 1953	14	6	2	0
Feb. 1, 1953	19	11	9	0
Feb. 17, 1953	22	19	14	3
Mar. 4, 1953	21	16	14	9
Mar. 14, 1953	24	20	20	17

Twenty young shoots with the first visible symptoms of basidiospore infections were selected and washed several times in sterile distilled water, then placed in quartz sand saturated with Hoagland's nutrient solution in 20 centimeter test tubes. Five tubes containing the test plants were placed in each of 4 controlled temperature cabinets at 5, 10, 15 and 20° C. Observations were made daily on the development of spermogonia and aecia by

checking the infected area on a dissecting microscope without removing the plants from the test tubes. Records were made (Table 3) of the date of the first appearance of spermatogonial exudate indicating the presence of mature spermatogonia, and of the first rupture of the epidermis by the aecia indicating the presence of mature aecia.

Development of both spermatogonia and aecia was most rapid at 20° C. At 5° C. development was greatly retarded and only 1 of 5 plants had mature aecia after a period of 40 days. Although infection by basidiospores may take place in the field during December and January, the development of spermatogonia and aecia is greatly retarded by the low temperatures generally prevailing then. This explains why mature aecia have not been observed before February 9, although basidiospore infections were found as early as December 13. Peltier (20, pp. 32-40) found a similar situation of retarded development to exist in the urediospore cycle of wheat stem rust subjected to low temperatures.

#### Aeciospore Cycle

Aeciospore production on spearmint in the field was observed in Indiana from April 15 to the first week in July by Baxter and Cummins (4). In Oregon viable aeciospores produced in the field have been collected from



Table 3. Influence of temperature on the development of spermogonia and aecia of mint rust.

Temperature	Plant number	Number of days until appearance of mature:	
		Spermogonia	Aecia
5° C.	1	33	--
	2	29	38
	3	--	--
	4	34	--
	5	30	--
10° C.	1	14	26
	2	19	30
	3	19	29
	4	16	26
	5	14	24
15° C.	1	7	14
	2	7	15
	3	9	16
	4	6	14
	5	10	16
20° C.	1	5	12
	2	5	11
	3	3	9
	4	4	9
	5	4	10

February 9 to June 27. Aeciospore production is heaviest during March and April. Although abundant aeciospores are produced in the field during late February and March, the earliest date recorded for the appearance of urediospores is April 22. The appearance of mature aecia on shoots collected in the field may be seen in Figure 3.

Vergovsky (27) stated that the incubation period from aeciospore infection to urediospores production was 12 to 15 days on peppermint. Niederhauser (18, p. 23) found an incubation period of 14 to 17 days for the production of urediospores from aeciospore inoculum on spearmint in the greenhouse.

To determine if aeciospores produced during February and March were capable of infecting peppermint under field conditions and under greenhouse conditions favorable for infection, field collections of aeciospores were made February 22, March 15 and 29 and April 22, 1952, and tested for percentage of viable spores by plating droplets of a spore suspension on 1.7 per cent water agar then counting the number of spores in 200 germinating after 24 hours incubation at 20° C. Peppermint plants about 6 inches high were inoculated by spraying with the spore suspensions, then placed in a moist chamber in the greenhouse at approximately 20° C. for 48 hours. Plants were removed from



Figure 3. Mature aecia on young peppermint shoots. Some shoots with multiple infections. Note hypertrophy, twisting and distortion, especially lower right.

the moist chamber and one lot of 5 plants was placed outside the greenhouse on the ground to simulate field conditions. A second lot of 5 plants was placed in the greenhouse at a mean temperature of 20° C. The plants were examined frequently and the incubation period necessary for the production of urediospores recorded. The incubation period under field conditions was greatly extended for aeciospore infections of February 22 and March 15 (Table 4), however, under greenhouse conditions favorable for infection and development of the mycelium, the incubation period was within the range previously reported by Vergovsky (27) and Niederhauser (18, p. 23).

Table 4. Comparison of the length of the incubation period for the production of mint rust urediospores under field and greenhouse conditions during February, March and April.

Date of spore collection	Per cent spore germination	Number of days incubation required before urediospores produced under:	
		Field conditions	Greenhouse conditions
Feb. 22	58.0	30*	15
Mar. 15	68.0	44	14
Mar. 29	72.0	20	15
Apr. 22	86.5	17	15

\*Plants in this test were lost after 30 days.

Peltier (20, p. 49) found the incubation period for the production of wheat rust urediospore to be a minimum of 8 weeks at a temperature of 0 to 5° C. Temperature seems to be the most important factor determining length of incubation but Raines (22) found a small increase in rust incubation time coincident with a decrease in host vigor.

The influence of temperature and storage time on aeciospore viability was studied. Young mint shoots bearing sporulating aecia were collected from the field during April 1952. Twenty shoots were placed in Petri dishes at each of 9 different temperatures. A temperature of minus 15° C. was maintained in a Philco deep freeze; minus 5° and 0° were maintained in freezing compartments of refrigerators and plus 5° to plus 30° C. were maintained in constant temperature cabinets. A variation in temperature of plus or minus 1.5° C. was the maximum variation recorded in any of the storage apparatus. At periodic intervals 4 shoots were selected from each storage temperature and the aeciospores removed by washing in 20 mls. of water to make a spore suspension. Droplets of the spore suspension were pipetted onto 1.7 per cent water agar in Petri plates and germinated at 20° C. The percentage germination of two hundred spores counted for each temperature is

recorded in Table 5.

Table 5. Effect of temperature and storage time on aeciospore viability.

Storage time in days	Percentage germination at temperatures* of:								
	-15	-5	0	+5	+10	+15	+20	+25	+30
1	0.0	0.0	1.0	46.5	43.5	59.5	65.5	32.0	40.5
2	0.0	0.0	0.0	54.0	61.5	81.5	86.0	30.0	1.5
4	-	-	-	52.0	44.5	53.5	83.0	25.5	0.5
7	-	-	-	63.5	85.0	13.5	41.5	0.5	0.0
14	-	-	-	45.5	46.5	2.0	11.0	2.0	0.0
28	-	-	-	16.5	9.0	2.0	0.0	0.0	0.0

\*plus or minus 1.5° C.

None of the aeciospores were viable after 1 day at -15 and -5° C., and only 1 per cent were viable at 0° C. Viability was lost rapidly at 25 and 30° C. but aeciospores held at 5, 10 and 15° C. were still viable after 28 days when the test was terminated.

Under field conditions environmental factors greatly influence aeciospore production, survival and infection. Several days of freezing weather beginning March 2, 1951 reduced aeciospore viability from 76 to 6 per cent. In exposed places rust mycelium and host cells associated



with it were killed by freezing weather during the first week of March, 1951 resulting in necrotic areas being formed at the point of aecial infection.

Beginning April 1, 1951, 27 consecutive days of clear, dry weather prevailed. Although abundant aeciospore inoculum was present no uredia were found until May 10, 13 days after a general rain. By May 17, uredial sori were abundant wherever sporulating aecia had been present on April 27.

The initial spread of mint rust by aeciospores has repeatedly been observed to be limited to a few feet. In 1953 Baxter and Cummins (4) reported no first-generation uredia at distances greater than 18 inches from the source of aeciospores.

Tests were conducted to determine the distance and general pattern of initial spread of mint rust by aeciospores and subsequent urediospores. On April 11, 1951, a mint field that had been fall-plowed was found to have a few widely scattered aecial sori. Six sporulating aecia were selected and marked. A careful examination of all mint shoots within a twelve-foot radius was made and no other aecial or uredial sori were found. These isolated aeciospore sources were carefully checked at about two-week intervals and the spread of rust from these sources

was charted. Fourteen days was considered as the minimum time necessary from infection to sporulation. Spread was determined by examination of all mint shoots within 12 feet of the original aeciospore source at 14 to 17 day intervals. The experiment was terminated June 28. Figure 4 is a representative chart showing distance and direction of spread. Initial spread by aeciospores was consistently less than 4 feet. Direction of spread consistently conformed to the general wind pattern in the test area.

#### Urediospore Cycle

Viable urediospores have been collected in the field from April 22 till December 13. Urediospores appearing in April and May have consistently been traced back to sporulating aecia in close proximity. Urediospores collected during December were not viable with the exception of those collected from foliage protected from weather extremes.

To determine if urediospores could overwinter on infected leaves under field conditions, heavily infected plants were collected during September, 1951 and air dried in a barn until October 18, then exposed to field conditions throughout the fall and winter. On October 20 a germination test showed 29.5 per cent viability of 400

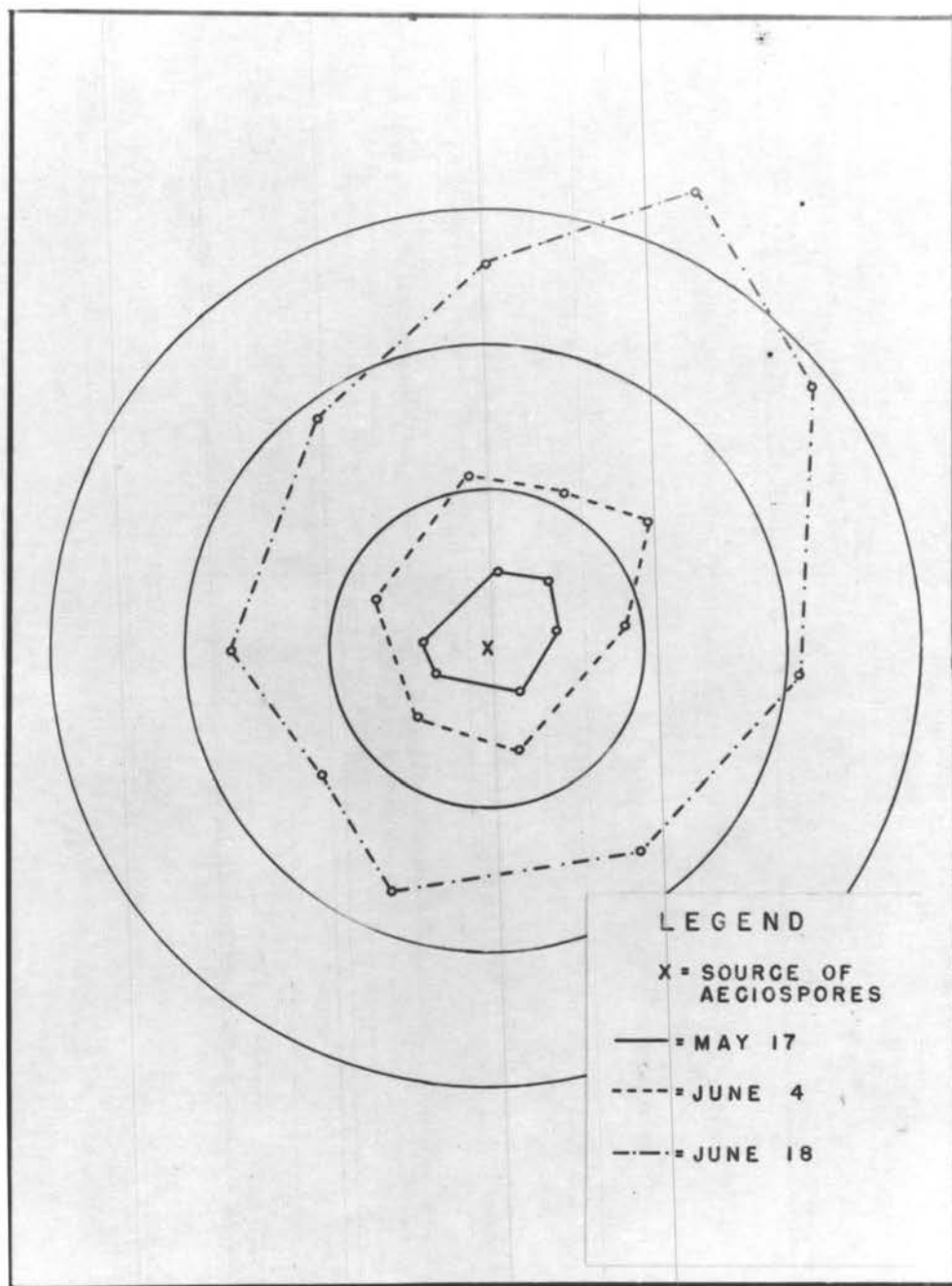


Figure 4. Rate and direction of spread of mint rust from a single aeciospore source. Each concentric circle represents 4 feet.

counted spores. Foliage of the heavily infected plants was placed in  $\frac{1}{4}$ -inch mesh wire baskets and the baskets placed in a mint field. Leaves were collected from the baskets on different dates from October 20, 1951 to January 24, 1952. The leaf samples were washed in water which was then strained through cheese cloth to remove the larger particles of plant debris. The spore suspension was pipetted onto 1.7 per cent water agar in Petri plates, the plates held at 20° C. for 24 hours, then spore counts were made and germination percentage recorded (Table 6).

Table 6. Viability of urediospores taken at different dates from mint foliage exposed to field conditions.

Date spores collected	Number of spores counted	Number of spores germinated	Per cent germination
Oct. 20, 1951	400	117	29.5
Nov. 19, 1951	200	34	17.0
Dec. 13, 1951	200	18	9.0
Dec. 23, 1951	200	9	4.5
Jan. 8, 1952	37	4	11.8
Jan. 23, 1952	86	0	0.

The time interval between the last viable urediospores found and their appearance on plants the succeeding season is 105 days. There is no evidence that urediospores overwinter in the field under Oregon conditions.

Increase and dissemination of the urediospore stage of mint rust is dependent on favorable climatic conditions. Vergovsky (27) found intense fructification followed 19 to 20 days after ideal meteorological conditions for infection. Field observations in Oregon have verified Vergovsky's observations. New "crops" of urediospores consistently appeared in large numbers 14 to 20 days after conditions favorable for infection. During July and August mint rust frequently increases so rapidly that an entire field with moderate rust infection may be 50 per cent defoliated 3 weeks after favorable conditions for infection.

Moisture has been observed to be the most important factor contributing to rapid increase of the urediospore stage of mint rust. Vergovsky (27) found that infection by urediospores did not take place in the absence of rain or dew.

It was repeatedly observed in the field that heavy, dense foliage favored infection of the lower leaves. The relative humidity under dense foliage was checked

periodically during July 1953 on a clear day and night. A hand aspirator psychrometer was used to measure relative humidity in these tests. Relative humidities were measured at or near the ground level, and at 6 and 12 inches above the ground level, both under dense foliage and in the open. Results are shown graphically in Figure 5.

In conjunction with the experiment on relative humidity in the field another experiment was conducted in the greenhouse to determine the relative humidity necessary for infection by urediospores. A humidity chamber was constructed as described by Hopp (10, pp. 25-44). It consisted of a glass chamber into which air was forced by a pump designed for aerating aquariums. Before entering the chamber the air was bubbled through distilled water or saturated salt solutions to acquire the desired relative humidity. Peppermint plants growing in 3-inch clay pots were inoculated by dusting rust urediospores on the leaves. The plants were placed in the humidity chamber for 48 hours then removed to the greenhouse bench and checked after 2 and 3 weeks for the presence of uredial sori. Since the relative humidity over salt solutions varies with the temperature (10, pp. 25-44), an attempt to keep a constant temperature of 20° C. was made by placing the test apparatus close to the greenhouse thermostat



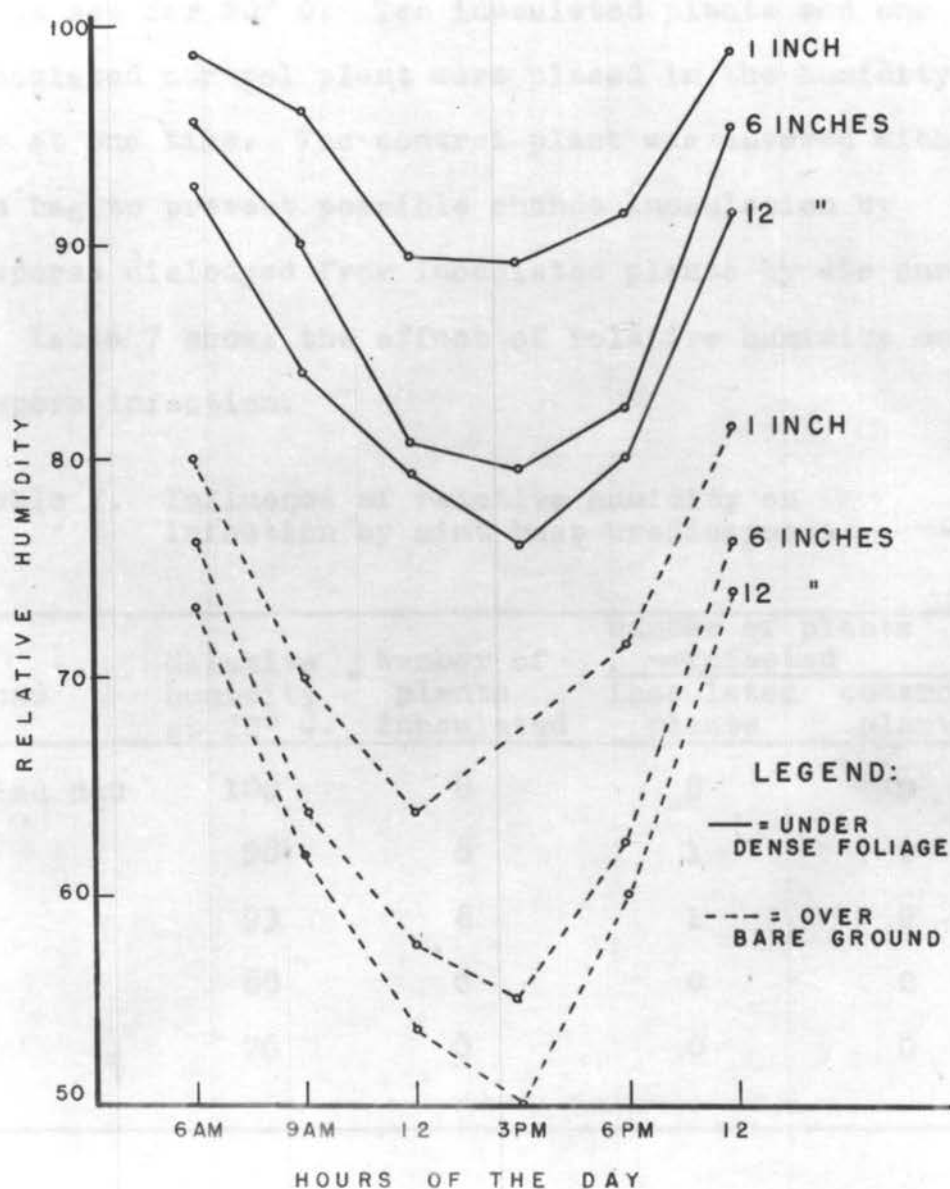


Figure 5. Comparison of relative humidity in the open and under dense mint foliage at different heights above-ground and at different hours of the day.

which was set for 20° C. Two inoculated plants and one non-inoculated control plant were placed in the humidity chamber at one time. The control plant was covered with a plastic bag to prevent possible chance inoculation by urediospores dislodged from inoculated plants by air currents. Table 7 shows the effect of relative humidity on urediospore infection.

Table 7. Influence of relative humidity on infection by mint rust urediospores.

Solutions used	Relative humidity at 20° C.	Number of plants inoculated	Number of plants infected	
			inoculated plants	control plants
Distilled H <sub>2</sub> O	100	8	8	0
K <sub>2</sub> SO <sub>4</sub>	98	8	1	0
KNO <sub>3</sub>	93	8	1	0
K <sub>2</sub> CrO <sub>4</sub>	88	6	0	0
NaCl	76	6	0	0

At 100 per cent relative humidity for 48 hours all inoculated test plants became infected and developed abundant uredial sori. Only 1 of 8 test plants was infected at 98 per cent relative humidity for 48 hours. The one test plant infected at 93 per cent relative humidity developed only 4 uredial sori while the test plant infected

at 98 per cent relative humidity had 12 sori. Because of the difficulty of maintaining an exact relative humidity on a transpiring leaf surface, it is probable that the infections at 98 and 93 per cent were the result of relative humidities higher than 98 per cent due to the proximity of stomata. Nevertheless, the data show that humidity near the saturation point is required for a high degree of infection by mint rust urediospores. As shown in Figure 5, the relative humidity under dense foliage during a clear day may be 98 per cent or more for as long as 6 hours. The absence of free air movement under dense mint foliage favors a high humidity for longer periods of time than in sparse mint cover. Conditions favorable for rust infection are thus more favorable in dense stands than in sparse stands. Field observations have demonstrated that the urediospore stage of mint rust starts sooner, increases more rapidly and is more severe in dense stands of mint than in sparse stands.

Radiation by direct sunlight, and associated dessication and high temperatures affect urediospore viability. Vergovsky (27) observed that urediospores collected from sun-dried peppermint leaves were not viable while urediospores collected from leaves dried in the shade remained viable.

To determine the effect of direct sunlight in the field on urediospore viability, a vigorous plant with heavy infection was selected and two of the lateral branches tied to stakes to expose the undersides of the leaves bearing numerous sporulating sori to direct radiation from the sun. Two other lateral branches were tied to a stake to expose only the upper leaf surfaces to direct sun radiation. The experiment was started on a clear day in July. Spores for germination tests were collected from exposed leaves after 2, 4 and 8 days of continuous clear weather. Spore suspensions were pipetted onto water agar in Petri plates, and after 24 hours incubation at 20° C. the percentage of viable spores was determined by counting 400 spores from each treatment.

The data in Table 8 shows spore germination reduced 42 per cent after 2 days, 65.7 per cent after 4 days, and 79.8 per cent after 8 days exposure to direct solar radiation. The viability of spores protected from direct solar radiation by the leaf was not affected. While solar radiation greatly reduces viability of urediospores from sori directly exposed to the sun, it is not an important factor in reducing urediospore viability in the field because nearly all sori are produced on the undersides of the leaves where they are not exposed to long durations of

direct sunlight.

Table 8. Comparison of the percentage of viable urediospores collected from sori exposed to and protected from direct solar radiation.

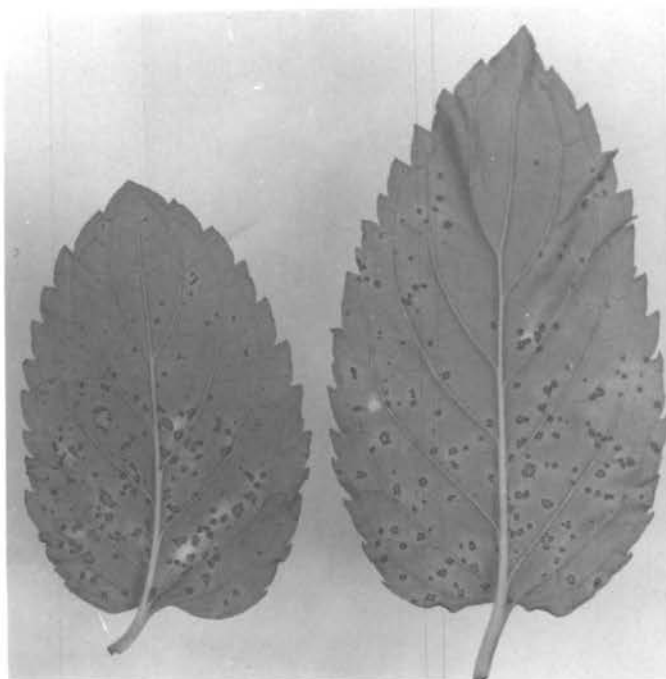
Duration of exposure	Per cent germination of 400 urediospores from sori:	
	Directly exposed to solar radiation	Protected from direct solar radiation
2 days	36.5	62.7
4 days	23.5	67.5
8 days	12.7	63.0

During periods of high temperature and light intensity rust mycelium and associated host cells are often killed resulting in small necrotic spots being formed on the leaves. Death of fungus and host tissue may take place either before or after rust sporulation. Figure 6 (A) shows 2 mint leaves with numerous necrotic spots collected in the field several days after the beginning of a hot, dry period during July. Although death of rust mycelium and associated host tissue may occur on plants growing under high soil moisture conditions, it occurs more often and to a greater degree on plants growing under moderate or low soil moisture conditions.

Death of rust mycelium and associated host cells has been experimentally induced by heat resulting in a



A



B

Figure 6. Death of rust infected tissue caused by high temperature. Leaves collected from the field (A); and leaves given 100° F. treatment for 4 hours 8 days after inoculation (B).



condition identical to that observed in the field after a period of hot, dry weather. A chamber designed and described by Owen (19, pp. 9-12) to maintain a minimum relative humidity of 40 per cent and a temperature from 80 to 150° F. was used. Test plants were inoculated with urediospores, placed in moist chambers for 48 hours then removed to the greenhouse bench. At periods of 4, 8 and 16 days after inoculation plants were placed in the heat chamber for varying lengths of time. Thermocouples and a Brown portable potentiometer were used to measure temperatures at the lower leaf surface. Thermocouples were made of number 36 copper and constantine wire joined with solder. A temperature variation of plus or minus 2° F. was consistently maintained by adjusting the distance of a Westinghouse infra-red lamp used as a heat source. The criterion used to determine whether rust mycelium and associated host tissue was killed consisted of the production of necrotic spots within 10 days after treatment. Figure 6 (B) shows the effect of 100° F. for 4 hours on leaves from a plant inoculated 8 days prior to heat treatment.

The data in Table 9 show that no death occurred at temperatures below 100° F. except in plants tested 16 days

Table 9. Effect of temperature, length of exposure and incubation time on death of rust mycelium and infected leaf cells.

Time after inoculation in days	Temperature in degrees Fahrenheit	Production of necrotic lesions indicating death after exposure for:		
		1 hr.	2 hrs.	4 hrs.
4	80	-	-	-
	90	-	-	-
	100	-	-	+
	110	-	-	+
	120	+	+	+
8	80	-	-	-
	90	-	-	-
	100	-	-	+
	110	-	+	+
	120	+	+	+
16	80	-	-	-
	90	-	-	+
	100	+	+	+
	110	+	+	+
	120	+	+	+

after inoculation and heated for 4 hours. Plants tested 16 days after inoculation had numerous sori that had broken through the leaf epidermis within the last 2 days. Death occurred at lower temperatures as the time after inoculation was increased. High temperatures for extended periods would effectively reduce rust severity in the field.

## CROP LOSS CAUSED BY PEPPERMINT RUST

All spore stages of Puccinia menthae cause some crop loss in peppermint either by direct parasitic action or indirectly by providing suitable infection courts for facultative parasites.

Losses Caused by Basidiospore  
Infection, Spermogonia and Aecia

Some mint shoots which become infected on the stem by basidiospores do not survive to produce aeciospores. Freezing weather may kill the infected area providing an entrance point for such facultative parasites as the Rhizoctonia stage of Pellicularia filamentosa (Pat.) Rogers, Fusarium spp., Phoma sp. and Pythium spp. The parasitic nature of the above fungi to peppermint has been established by wound inoculation and subsequent reisolation (11).

Mint shoots which become infected after plowing and have large aecial lesions on their stems rarely survive to maturity. Vergovsky (27) reported that young shoots with aecia died from desiccation of the infected area when growing under low soil moisture conditions. Under high soil moisture, hypertrophy was induced thus preventing desiccation.

Under field conditions in Oregon, shoots with aecial infections rarely die from desiccation. More frequently death is the result of invasion of the sporulating aecial pustule by facultative parasites. In addition to the fungi previously mentioned, slugs frequently eat the entire infected area on stems and leaves.

On April 14, 1952 four unplowed mint fields were examined and found to have 72, 84, 91 and 95 per cent respectively of the new shoots infected with the aecial stage of mint rust. Two hundred shoots were examined in each field in determining the above percentages. An analysis of the number of shoots killed by the aeciospore stage in these fields was made by critically examining an area of one square yard in each field and recording the total number of shoots counted and the number killed (Table 10). The test was not continued to determine how many shoots would eventually be killed because the fields were plowed soon after the first counts were made.

Isolations were made from 5 recently killed shoots from each of the four fields and from several live shoots bearing aecial lesions that appeared to be invaded by secondary organisms. Fungi belonging to the genera Fusarium and Phoma were the predominant organisms isolated. Both these genera are known to contain species that are

facultative parasites.

Table 10. The percentage of peppermint shoots killed in four different fields from invasion of the aecial lesions by secondary organisms.

Field number	Number of shoots examined	Number of shoots killed	Per cent killed
1	286	29	10.1
2	344	46	13.4
3	186	22	11.8
4	294	40	13.6

Peppermint in fields with 90 per cent or more of the shoots bearing aecial lesions showed only a slight reduction in stand after plowing when compared to peppermint with 20 per cent of the shoots bearing aecial lesions. Twenty infected shoots produced a stand of 17 plants, while 20 healthy shoots produced a stand of 20 plants when planted four inches deep in field soil in flats in the greenhouse. The infected shoots readily rooted above the point of infection and produced healthy plants except in 3 cases in which the infected shoots failed to survive.



### Losses Caused by the Urediospore Stage

The most apparent loss caused by urediospores is leaf drop. Actual reduction in yield from other, less apparent causes may equal or exceed losses from leaf drop. In 1951 field tests were conducted that demonstrated yield could be reduced nearly 30 per cent by the urediospore stage without leaf drop.

During the summer of 1951 rust increase was cyclic according to periods of weather conditions favorable for infection. Heavily infested areas appeared in fields near the points of initial infection by aeciospores. It was thus possible to take yield data from severely and adjacent lightly or non-infested areas in the same field.

In a preliminary experiment comparative leaf drop was determined on heavily and lightly infected mint. Two areas 20 feet square with uniformly heavy and light rust infestation, respectively, were staked. Each area was further subdivided into 25 plots 4 feet square. One plant from each small plot was selected by entering the plot and cutting a plant at the soil level. The number of pairs of leaves that had dropped from the main stem was recorded for each plant. A comparison of the number of pairs of leaves lost on heavily and lightly infected plants is given in Table 11.

Table 11. Comparison of leaf drop from heavily and lightly rust infected peppermint plants.

Plant number	Number of pairs of leaves dropped from the main stem of plants with:	
	Heavy rust infection	Light rust infection
1	8.5	6.0
2	7.0	4.0
3	8.5	5.5
4	5.0	6.5
5	6.0	3.0
6	5.5	5.0
7	7.5	4.5
8	7.5	4.0
9	8.0	3.5
10	6.5	5.5
11	9.0	4.0
12	6.0	3.5
13	6.5	7.0
14	8.5	3.5
15	7.5	4.5
16	7.0	5.0
17	5.5	4.0
18	8.0	4.5
19	8.5	6.5
20	7.0	3.0
21	9.0	4.5
22	6.5	4.0
23	6.0	5.0
24	8.0	3.5
25	6.0	4.0
total	179.0	total 114.0
mean	7.2	mean 4.6

The same plots used for leaf drop comparisons were cut, the green hay weighed, a sample removed for dry weight determination and the remaining hay distilled to determine yield of oil. After determining the dry weight of the hay the percentage of oil of the dry weight was

calculated. These data are presented in Table 12.

Table 12. Comparison of oil yield from heavily and lightly rust infected peppermint.

Rust rating	Green wt. of hay in pounds	Dry wt. of hay in pounds	Oil yield in grams	Per cent oil of the dry wt.
Heavy	116	24.8	106	0.94
Light	110	23.5	136	1.27

The oil yield from heavily rust infected plants was 26 per cent less than from lightly infected plants when calculated on the basis of percentage of oil of the dry hay. It is hardly possible that the small difference in leaf drop shown in Table 11 could account for 26 per cent reduction in oil yield.

In another test an attempt was made to determine how much reduction in yield might be attributed to rust on the leaves exclusive of leaf drop. Peppermint from adjacent heavily and lightly rusted plots in three different fields was cut 8 inches above the soil level to eliminate the leaf drop zone. Green weight, dry weight, oil yield and percentage of oil of the dry weight were determined as previously described, and are given in Table 13.

Table 13. Comparison of oil yield from heavily and lightly rust infected peppermint from 3 different fields.

Rust infection and field no.	Green wt. of hay in pounds	Dry wt. of hay in pounds	Oil yield in grams	Per cent oil of the dry wt.
1	75	16.05	64	0.88
Heavy 2	85	18.19	68	0.82
3	70	14.98	64	0.94
1	79	16.90	96	1.25
Light 2	65	13.91	73	1.15
3	83	17.76	94	1.17

The per cent reduction in yield of heavily rust infected compared with lightly rust infected mint was 29.6, 28.7 and 19.7 for the three fields respectively, when calculated on a basis of percentage of oil of the dry weight.

Heavy urediospore infection inhibits lateral stem development, especially when heavy infection occurs early in plant development. Figure 7 shows a comparison of lateral stem development on rust free (left) and heavily infected (right) plants grown under similar stand density and environmental conditions.

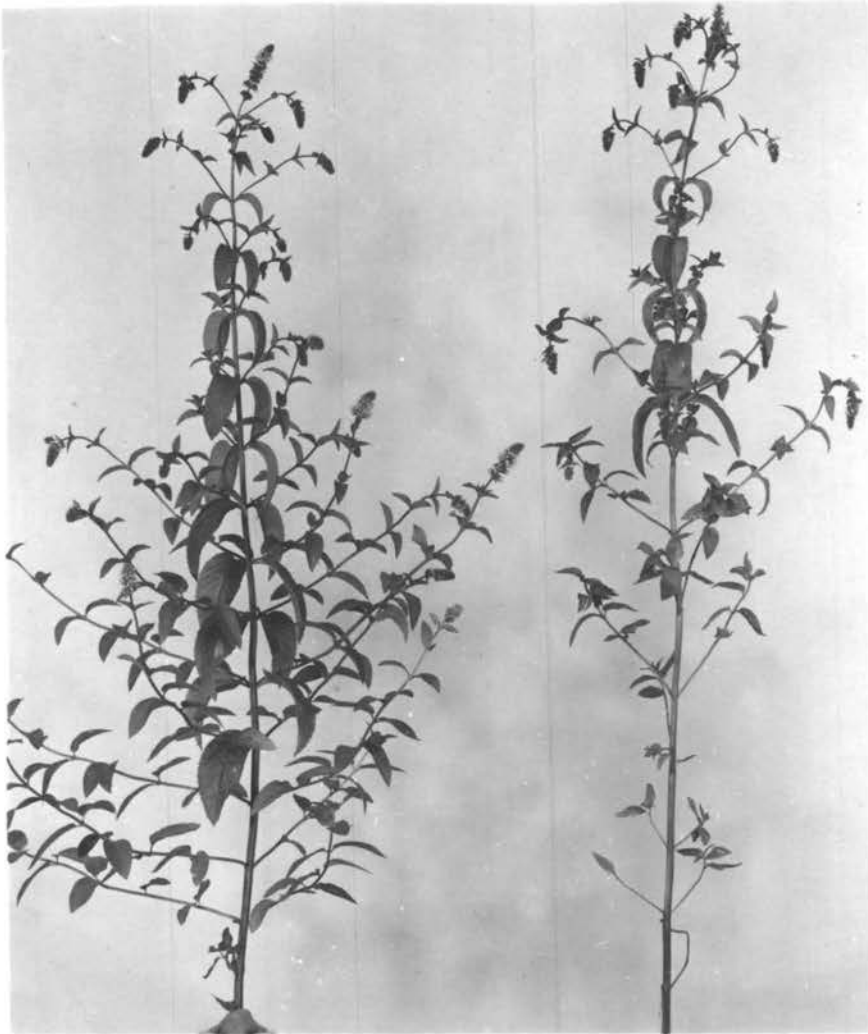


Figure 7. Comparison of leaf drop and lateral stem development on healthy (left) and severely rust infected (right) peppermint plants.

Heavy urediospore infection reduces the production of rhizomes and those produced are weaker and more susceptible to root and rhizome rots (11). To ascertain how much rhizome production might be reduced, two greenhouse beds were filled with similar soil and planted with 10 plants each. The plants used in this test were rooted cuttings of uniform size and vigor from the same clone and were grown under similar environmental conditions. Plants in one bed were inoculated with rust on June 1 and thereafter at 2 week intervals. Plants in the other bed were maintained rust free. The mint in both beds was cut when the first bloom appeared. The regrowth from the rust infected plants was again heavily inoculated with rust. Plants from both beds were dug in September and the rhizomes compared by number, size, and weight per plant. These data are given in Table 14.

A statistical analysis was made to determine if the difference between the observed means was significant at the 5 per cent level. The hypothesis that the population mean of the differences is equal to zero was tested by the t-test for each quantitative character measured. There was no significant difference in the number of rhizomes produced by rust infected and rust free plants. The mean values of diameter, length and weight of rhizomes produced



by rust free plants were significantly greater than the mean values from rhizomes produced by rust infected plants.

Table 14. Comparison of number, size and weight of rhizomes produced by heavily rust infected and rust free peppermint plants.

Rust class and plant number	Number of rhizomes produced	Average diameter of rhizomes in millimeters	Average length of rhizomes in inches	Dry weight of rhizomes in grams	
Rust infected plants	1	6	3.5	21	7.2
	2	4	4.0	23	6.4
	3	4	4.5	17	6.1
	4	6	4.0	26	8.2
	5	6	3.5	23	7.1
	6	5	3.5	18	4.9
	7	4	5.0	20	6.5
	8	7	3.0	23	8.3
	9	5	3.5	21	6.3
	10	5	3.5	20	6.0
Sum Mean	52 5.2	Sum Mean 38.0 3.8	Sum Mean 212 21.2	Sum Mean 67 6.7	
Rust free plants	1	5	5.5	30	11.2
	2	4	5.0	27	9.2
	3	5	5.0	24	9.0
	4	6	6.0	26	10.3
	5	6	5.0	29	9.2
	6	6	6.0	30	12.4
	7	5	6.5	32	12.6
	8	6	5.5	29	11.8
	9	8	4.5	24	10.0
	10	6	5.5	28	11.0
Sum Mean	57 5.7	Sum Mean 54.5 5.5	Sum Mean 279 27.9	Sum Mean 106.7 10.7	

### Losses Caused by the Teliospore Cycle

Telial sori on rhizomes function as points of entry for facultative parasites that cause root and rhizome rots. Rhizome rots caused by Rhizoctonia solani, Fusarium spp. Pythium sp. and Phoma sp. (11) have repeatedly been observed to start from rust pustules on the rhizomes. Serious reductions in stand have been observed in fields where rhizome rots were severe.

## METHODS OF EVALUATING RUST SEVERITY AND CONTROL DATA

## Evaluation of Rust Severity

Taking accurate yield data from large numbers of plots is difficult and time consuming. For example, an experiment testing the relative rust control of 10 fungicides at 3 different times of application with 4 replications would involve the separate cutting, weighing and distillation of more than 100 plots. The average distillation time for each plot is 45 minutes to 1 hour. During the interval of 10 or more days required to complete distillation such factors as rust increase and maturity could greatly influence yield thus making comparisons of yield from plots harvested at the beginning and end unreliable. In order to circumvent this situation a criterion was needed that would be both rapid and directly correlated with yield.

Leaf drop and recording the amount of rust on the plants were both considered. Since leaf drop had previously been shown to account for a lesser amount of the reduction in yield than had rust on the leaves, the latter was chosen for further evaluation.

Bullis et al (5, p. 10) state that approximately 99 per cent of the oil bearing glands of Mentha piperita are

on the leaves. More than 95 per cent of uredial sori are also on the leaf surfaces. When uredial sori erupt they break the host epidermis and destroy the oil glands situated on that part of the epidermis (Figure 8). Later, desiccation of the infected area around the sori results in further reduction of oil gland numbers.

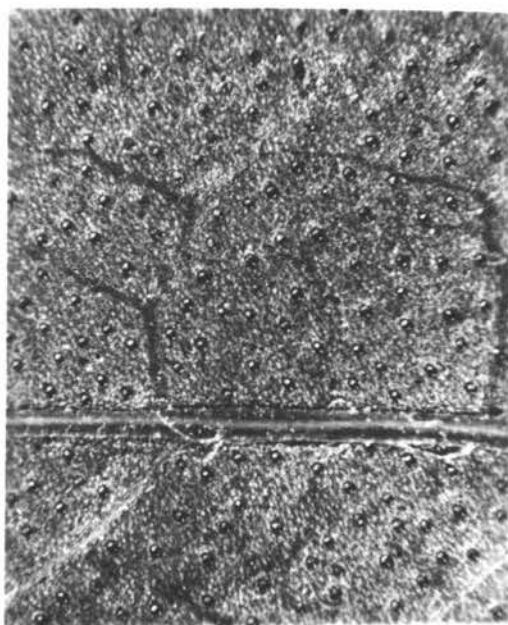
It was conceivable that disease classes based on the percentage of oil glands destroyed on the leaves would be a reliable criterion of yield losses. The reliability of using disease classes to compute an "infection" or "disease index" has been well established by McKinney (15) and applied with good results to other leaf diseases (12).

Horsfall and Heuberger (12) further extended the application of the McKinney infection-index formula. Marsh et al (13) found that the method was open to statistical analysis.

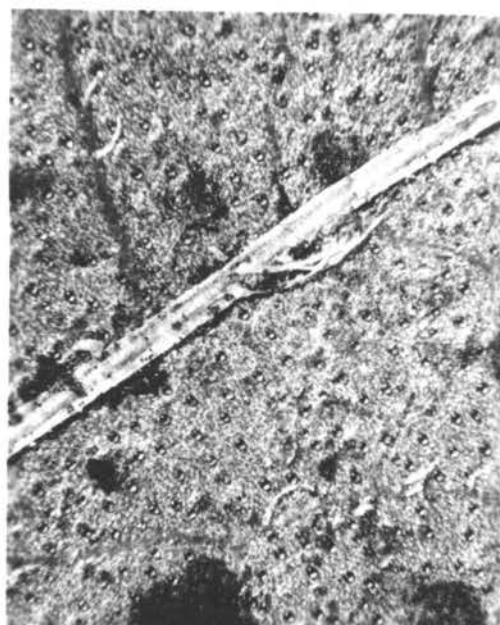
The method as applied by Horsfall and Heuberger (12) consists of having classes of disease representative of different percentages of infection. The mean disease index for a plot can be computed by the following formula:

$$\text{Disease index} = \frac{\text{sum of class numbers}}{NT}$$

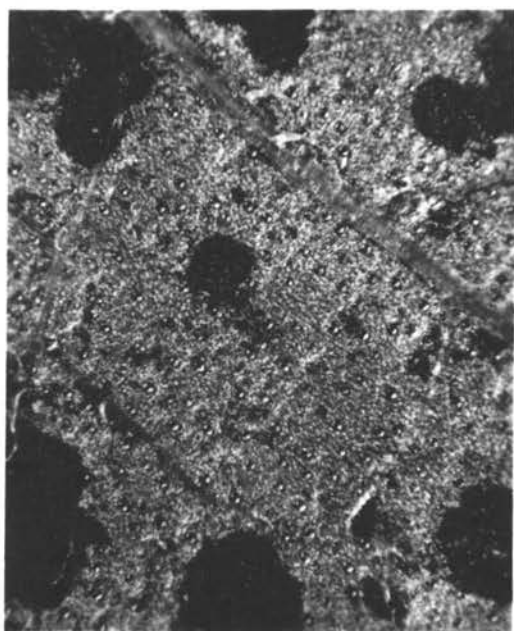
The value T is the total number of plants or leaves rated and N is the number of the highest disease class.



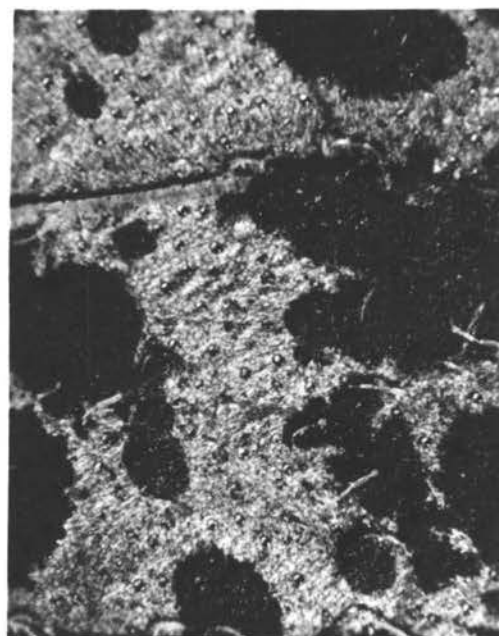
A



B



C



D

Figure 8. Destruction of oil glands on leaf surfaces by different degrees of rust. A, none; B, light; C, moderate; D, severe rust infection.



The disease index is the mean infection index for plants or leaves. In peppermint, a disease index based on percentage of oil glands destroyed should be a reliable criterion of yield.

To determine the percentage of oil glands destroyed by erupting rust sori fifteen peppermint plants were grown from uniform rooted cutting in 6 inch clay pots. When the plants were 16 to 18 inches high one leaf of each pair on the main stem was covered with folded paper squares held in place with paper clips. The 15 plants were then divided into 3 lots of 5 plants each. The uncovered leaves of each plant were sprayed with urediospore suspensions of different concentrations of spores. Plants in the 3 lots received an inoculum of 50,000, 10,000 and 1,000 urediospores per ml. of suspension respectively. After the plants were dry the paper squares were removed and the plants were placed in a moist chamber for 72 hours, then returned to the greenhouse bench. Three weeks after inoculation, paired leaves from the base, middle and upper parts of the stem were separately collected, pressed and dried. Forty-five pairs of leaves were thus available for analyses. However, considerable variation in the amount of infection on leaves sprayed with the same spore suspension occurred. Because of this variation, leaves representing light, moderate and



heavy rust infection with their rust free counterparts were arbitrarily chosen for analysis of leaf gland numbers.

Oil glands were counted with a compound microscope fitted with a 5 x objective and a 10 x ocular containing a counting grid. Five counts were made at random on the underside of the leaf blade and the number of oil glands in each microscopic field was recorded (Table 15).

The percentage reduction in oil gland numbers for the different disease classes was computed and is given in Table 15. Rust infection classes 1, 2 and 3 reduced oil gland numbers from 2.46 to 4.66, 16.74 to 22.13, and 39.91 to 48.14 per cent respectively.

Disease classes of 0, 1, 2 and 3 could be representative of 0, 0-5, 6-25 and 26-50 per cent reduction in oil gland number respectively. Then by application of the formula:

$$\text{Per cent reduction in oil glands} = \frac{(X 0) + (X 1) + (X 2) + (X 3)}{3 T} \times 50 = \text{disease index}$$

where X 0 = the number of leaves in rust class 0  
 X 1 = " " " " " " " 1  
 X 2 = " " " " " " " 2  
 X 3 = " " " " " " " 3

3 = numerical value of the highest rust class.

T = total number of leaves counted.

The value 50 rather than 100 is used to transform the

Table 15. Number of oil glands per unit area on peppermint leaves with four classes of rust infection and from three positions on the plant.

Position on plant	Rust classes	Number of oil glands per microscope field					Totals of fields by disease classes	Means of fields by disease classes*	Per cent reduction in glands by disease classes
		1	2	3	4	5			
Base	0	63	66	71	58	64	322	64.4	0.0
	1	58	61	66	57	65	307	61.4	4.66
	2	53	51	49	47	52	252	50.4	21.74
	3	33	35	38	32	29	167	33.4	48.14
Middle	0	73	74	68	77	74	366	73.2	0.0
	1	66	71	70	73	77	357	71.4	2.46
	2	55	59	57	54	60	285	57.0	22.13
	3	48	42	44	39	40	213	42.6	41.80
Top	0	99	92	89	92	94	466	93.2	0.0
	1	88	82	91	90	96	447	89.4	4.08
	2	76	80	82	73	77	388	77.6	16.74
	3	58	63	52	51	56	280	56.0	39.91

\*L.S.D. at .05 for means of disease classes 1, 2 and 3 = 5.20, 6.51 and 8.13 for base, middle and top respectively. L.S.D. for position means of rust class 0 = 8.17.

data to a percentage basis because in no case have leaves been observed with more than 50 per cent of the oil glands destroyed by rust.

If a disease rating method were a reliable criterion of yield much of the labor and experimental error of taking yield data could be circumvented. An experiment was designed to test the reliability of using the disease rating described above rather taking yield data. Leaves with rust ratings 0, 1, 2 and 3 from each of 3 positions on the main stem of mint plants were mounted under a transparent plastic cover to use as a standard for comparison in selecting leaves in the field. Figure 9 shows the standard used. A large sample of leaves was collected for each rust rating from the lowest pair of leaves on the main stem, the fifth pair above the lowest, and the tenth pair above the lowest pair. The samples were air dried for two weeks then 100 gram samples were distilled in the laboratory using standard taper flasks and condensers. The distillate was collected in a titration burette and oil yield read directly from the burette to the nearest .1 and estimated to the nearest .05 milliliters. The oil yield from each sample is given in Table 16.

Although the data in Table 16 are not subject to statistical analysis because of the absence of replications

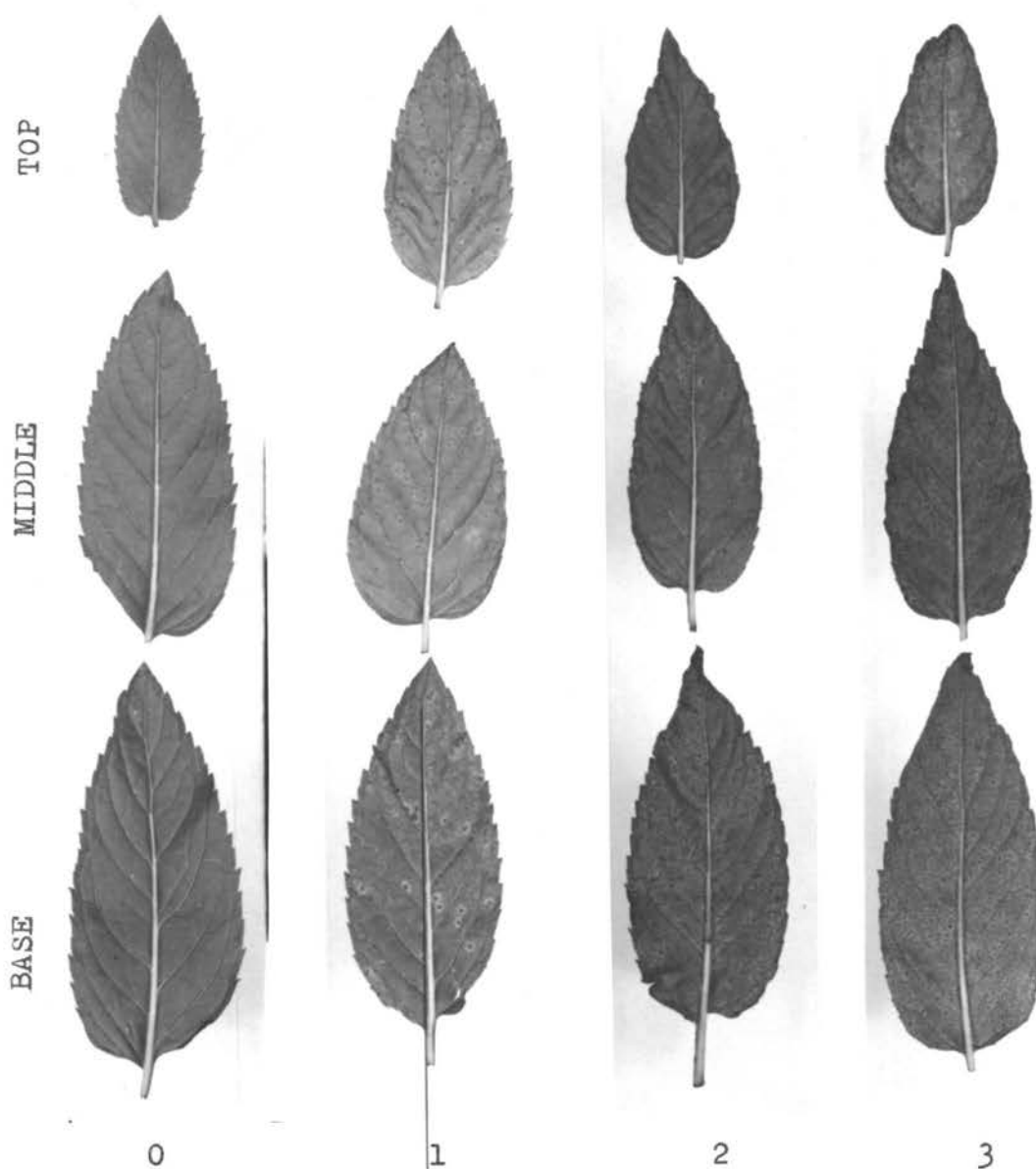


Figure 9. Rust rating standard used to evaluate rust severity and control data. Rust classes 0, 1, 2 and 3 represent 0, 0-5, 6-25 and 26-50 per cent reduction in oil gland numbers respectively.

certain trends are apparent. The oil yield consistently decreased as rust severity increased. The percentage reduction in yield for the rust severity classes in each case is within the arbitrary values of 0, 0-5, 6-25 and 26-50 per cent for classes 0, 1, 2 and 3 respectively. The oil yield consistently increased in leaves taken from base to top (Table 16) as did oil gland numbers (Table 15).

Table 16. Yield of oil from peppermint leaves with four degrees of rust infection collected from 3 positions on the plants.

Position on plant	Rust classes	Oil yield in mls. from 100 grams of leaves	Per cent reduction in yield
Base	0	2.25	0.0
	1	2.15	4.4
	2	2.00	11.1
	3	1.55	31.1
Middle	0	3.05	0.0
	1	*	---
	2	2.50	18.0
	3	1.85	39.3
Top	0	3.35	0.0
	1	3.30	1.5
	2	3.00	10.4
	3	2.15	35.8

\*Yield was lost by an accident.



### Evaluation of Control

The reliability of using a disease index as a criterion of rust severity and yield in evaluating the relative effectiveness of different fungicides was tested in the field. The details of this experiment are given in the section on control pp. 77 to 82. Table 27, p. 82 shows a comparison of disease index and yield from plots treated with 10 different fungicides. The correlation coefficient ( $r$ ) of disease index x yield data from 10 fungicide treatments was calculated and is shown in Table 17. It can be seen from the data in Table 27 that as disease index increases, yield decreases which is to be expected since disease index measures the amount of oil glands destroyed by erupting rust sori.

Table 17. Statistical analysis of the correlation of disease index and yield from 10 fungicide treatments.

Source of variation	SS	SS	$\sqrt{\frac{SS}{SS}}$	r	De- grees free- dom of r	r at t .05
	x	y	x y			
Replication	75,458.2105	274.6966	.6031	3	.8783	
Treatment	2,884,474.0359	1,698.3739	-.9417	9	.6021	
Error	1,286,602.3598	1,134.2850	-.2776	39	.3044	
Total	9,995,259.3018	3,161.5280	-.5531	53	-----	



## CONTROL OF PEPPERMINT RUST

The control of rust diseases of field crops has been best achieved by breeding and selection of resistant varieties, and, in the case of heteroecious rusts, by eradication of alternate hosts. Puccinia menthae is an autoecious rust, and the development of resistant varieties by breeding or selection usually takes several years. Because of the immediate need other methods of control were investigated.

### Cultural Practices

Vergovsky (27) established that Puccinia menthae was not systemic in peppermint. He found that infected shoots and rhizomes would produce healthy plants if covered with 5 cm. or more of soil. Infected shoots covered with less than 4 cm. of soil produced infected plants because the sporulating infected area was carried above the soil surface by shoot elongation. Vergovsky's results have been verified for Oregon conditions by repeated field observations. Vergovsky suggested deep fall plowing as a means of control.

In 1951 Steenland (26) and Dietz et al. (7) reported

that the incidence of the aecial stage of mint rust was much higher on back-furrows, dead-furrows and head-lands where old stubble carrying teliospore inoculum was not covered by plowing. Clean fall plowing was suggested for control (26), (7). In 1953 Baxter and Cummins (4) reiterated the value of deep fall plowing and elimination of plant debris along back-furrows and dead-furrows although no experimental data were given.

A comparison was made of the incidence of the aecial stage on unplowed, poorly-plowed and thoroughly plowed portions of the same mint field. The experiment was conducted as part of a demonstration for growers. Table 18 shows a comparison of the incidence of the aecial stage from 3 plowing practices.

The data show that thorough coverage of plant debris during plowing greatly reduces the incidence of the aecial stage, but may not completely eliminate it. The data bear out field observations that most rust starting in the field originates on poorly-plowed areas such as dead-furrows, back-furrows and head-lands.

After plowing the incidence of functional aecia can be reduced still more by cultivation. Mint shoots with aecia are very brittle at the point of infection and are readily broken. Cultivation with a finger or rotary hoe

Table 18. Effect of different plowing practices on the incidence of the aecial stage of mint rust.

Plot number	Per cent of 500 shoots bearing aecial pustules on mint that was:		
	Not plowed	Poorly-plowed*	Thoroughly plowed**
1	91.0	6.0	0.8
2	72.0	3.4	0.0
3	84.0	4.4	0.0

\* Poorly-plowed indicates that considerable plant debris from the previous seasons growth remained uncovered after plowing.

\*\*Thoroughly plowed indicates that most all plant debris was covered by plowing.

type weeders breaks off many of the infected shoots. When the infected shoots are broken before the aecial pustules have released spores, the broken shoots normally die back to the first node below the break and the aecial pustule may not become functional.

A comparison was made of the effect of cultivation in reducing the number of infected shoots bearing aecia. Twenty mint shoots with aecia which had not yet released spores were marked in each of three plots. A finger type weeder was drawn over the plots and the number of infected shoots that were broken off was recorded. Ten days later

the plots were checked again and the number of shoots that had survived to produce aeciospores was recorded. These data are given in Table 19.

Table 19. Effect of cultivation on the survival of aecial infected peppermint shoots.

Plot number	Number of aecial infected shoots:		
	Present before cultivation	Broken off by cultivation	Functional 10 days after cultivation
1	20	11	13
2	20	9	12
3	20	8	9

The data in Table 19 show that approximately half the infected shoots were broken off by cultivation, of which only 6 out of 28 survived to produce aeciospores. The data show that cultivation reduces the number of aecial infections that could become functional in rust spread.

Since most of the mint grown in Oregon is irrigated by overhead sprinkler systems frequent irrigation might provide moisture conditions favorable for rust infection and result in rapid increase of the disease to destructive proportions. However, observations made in the field in 1951 indicated that although rust might be more abundant

on irrigated mint, losses from rust were greater on non-irrigated mint. In 1952 a field experiment was conducted to test the effect of irrigation on rust severity and leaf drop.

A series of paired plots were established by removing every other pair of sprinklers on an irrigation line. The plots were 10 feet square and located in the central part of either the irrigated or non-irrigated sections. The amount of leaf drop on the main stem and a computed disease index were used as criteria of rust severity. Plots were sampled by cutting 10 plants at random in each plot. The leaf drop and disease rating of each plant was recorded. Leaf drop and disease index values in replication are recorded in Table 20.

A statistical analysis of the data was made by the method of paired observations. The computed t-value for leaf drop was 12.86 with 4 degrees of freedom. The critical t-value at the 1 per cent level of significance is 4.60, therefore one may conclude that irrigation reduces leaf drop significantly. The computed t-value for disease index was 0.35187 with 4 degrees freedom and the critical t-value is 4.60 at the 1 per cent level. Therefore, irrigation did not significantly increase disease severity.



Table 20. Effect of irrigation on rust severity and leaf drop.

Replication number	Irrigated		Not irrigated	
	Leaf drop*	Disease index*	Leaf drop*	Disease index*
1	6.4	1.50	9.9	1.37
2	6.0	1.53	10.3	1.27
3	6.1	1.57	10.1	1.40
4	5.8	1.37	10.0	1.43
5	6.0	1.73	9.0	1.67

\*Each value is the mean of 10 observations.

Dimock and Baker (8) have demonstrated that rust on snapdragons resulted in more host damage under semiarid conditions than under humid conditions because of excessive desiccation of host tissue brought about by low humidity. A similar situation exists on irrigated and non-irrigated mint in Oregon, where low soil moisture appears to be one of the most significant factor associated with desiccation of rust infected mint leaves.

#### Eradication of Wild and Escaped Mint

Several species of the family Labiatae that are hosts for Puccinia menthae grow wild in Oregon. Wild species of the Labiatae that have been demonstrated to be hosts for



the race of rust attacking commercial peppermint in Oregon are Mentha canadensis L., M. piperita L. var. American and Satureja douglasii (Benth) Brig. Two of the above species, M. piperita var. American and M. canadensis, have been observed to act as sources of rust inoculum for adjacent peppermint fields. It is not likely that S. douglasii is an important source of rust inoculum since it has rarely been found near peppermint fields and most specimens collected were not infected with rust.

Peppermint which has escaped from cultivation has been observed to be an important source of rust inoculum for adjacent commercial plantings. Escaped mint is common along field edges, creeks, sloughs and ditchbanks in the mint growing areas. Such escaped mint is nearly 100 per cent infected with the aecial stage of mint rust in the spring and functions as an important source of inoculum for commercial plantings.

Trials were undertaken to determine the most feasible and economical methods of preventing spread of rust from wild and escaped mints to commercial plantings. Eradication of rust infected wild and escaped mints, to be effective in preventing rust spread, must be accomplished before or during aeciospore discharge in the spring. Attempts by growers to kill rust infected, escaped mint

with 2,4-D (2,4-Dimethylamine) indicated that the infected plants were killed too slowly to prevent rust spread. It seemed desirable to test chemicals that would cause an initial quick kill of the infected plant parts to prevent initial rust spread followed by systemic herbicides such as 2,4-D for complete eradication.

In 1951 8 materials and combinations were used in a replicated field test to determine their effectiveness for killing the above-ground parts of rust infected plants before aeciospore discharge. Table 21 gives the materials, rates and relative effectiveness in killing plant parts before spore discharge. The materials were rated as excellent, good, fair and poor. All materials were applied as a spray and the test plots received the equivalent of 100 gallons per acre.

The most consistent results were obtained with the dinitro weed-killers in diesel oil or in diesel oil and water. Later tests and widespread usage by growers have further substantiated the effectiveness of dinitro weed-killers in diesel oil. 2,4-D alone and 2,4-D plus 2,4,5-T were not effective in killing the mint plants before infected plants had discharged viable spores. However the contact weed-killers, such as dinitro, are not effective in eradicating the wild and escaped mint. Good regrowth

Table 21. Relative effectiveness of different herbicides for killing rust infected mint plants before aeciospore discharge.

Materials	Rates per acre	Effectiveness rating
Sodium cyanate 80%	20 lbs. in 100 gal. water	Good
Potassium cyanate 91%	20 lbs. in 100 gal. water	Good
Dinitro ortho secondary butyl Phenol 55%	1½ quarts in 100 gal. water	Fair
Dinitro-o-sec. butyl phenol Plus diesel oil	1½ quarts and 30 gal. in 70 gal. water	Good
Dinitro-o-sec. butyl phenol Plus diesel oil	1½ quarts in 100 gal. diesel oil	Excellent
Diesel oil	100 gal.	Good
2,4-Dimethylamine	1 lb. parent acid in 100 gal. water	Poor
2,4-Dimethylamine plus 2,4,5-Triethylamine	1 lb. parent acid each in 100 gal. water	Fair

nearly always appears after infected plants have been burned back by dinitro and diesel oil, but this regrowth is not infected with rust. A spray mixture of equal parts of 2,4-D and 2,4,5-T usually results in complete eradication of the regrowth when applied during warm days in July.

In some cases 2 sprays of the mixture have been necessary for a complete kill.

#### Eradication of the Aecial Stage by Chemicals

The aecial stage of mint rust is present in Oregon mint fields from March to May wherever plowing did not completely cover the previous season's stubble and rhizomes. If the aecial stage could be eradicated from infected shoots in the field and from adjacent wild and escaped mint, the field should remain rust free until infected by wind-borne inoculum from adjacent infected areas. Heavy infection from wind-borne urediospores takes place late in the growing season and usually does not result in severe losses. Several trials were conducted in 1951 to determine if eradication of the aecial stage could be accomplished before spore dissemination.

The trials consisted of applying contact plant-killer chemicals to the mint when the new shoots were 3 to 5 inches high thus killing back all above ground growth. In a preliminary trial to determine the most effective of several materials 8 different materials or mixtures were applied as sprays or dusts to unreplicated field plots 30 feet wide and 340 feet long. As many aecial infected shoots as could be found were marked in each plot before

treatment. Ten days after treatment the marked shoots were examined and the number still having functional aecia recorded. About 10 days before harvest plants in the plots were rated as lightly, moderately or severely rust infected. The data from this preliminary trial are recorded in Table 22.

On the basis of effective eradication of the aecial stage and minimum permanent damage to the stand, the mixture of dinitro plus cyanate in water and the mixture of dinitro and diesel oil in water were chosen for further trials. Although dinitro in diesel oil alone resulted in complete eradication of infected shoots, it also resulted in a severe reduction in stand. All other treated plots had stands judged equal to the check plot at harvest-time.

The two materials selected as the best in the 1951 trial were further tested in 1952. A randomized block design consisting of 3 replications was employed. Criteria were: (1) eradication within 10 days after treatment, (2) leaf drop from the main stem at harvest time, (3) a computed disease index at harvest time, and (4) stand density 6 weeks after treatment. Leaf drop, disease index and stand density are subject to statistical analysis. Table 23 gives the data on eradication, leaf drop and disease index. Table 24 shows a comparison of stand counts on treated and untreated plots 6 weeks after treatment.



Table 22. Effectiveness of 8 treatments for the eradication of the aecial stage of mint rust.

Treatment	Rate per acre	Number of infected shoots:		Rust rating 10 days before harvest
		Before treatment	After treatment	
Potassium cyanate (91%) spray	15 lbs. in 100 gal. water	11	4	Moderate
Sodium cyanate (40%) spray	40 lbs. in 100 gal. water	15	2	Moderate
Potassium cyanate (91%) plus dinitro spray	15 lbs. and $1\frac{1}{2}$ qts. in 100 gal. water	16	0	Light
Dinitro plus diesel oil spray	$1\frac{1}{2}$ qts. in 50 gal. diesel	11	0	Light
Dinitro plus diesel oil spray	$1\frac{1}{2}$ qts. and 20 gal. in 100 gal. water	19	0	Light
Sodium cyanamid dust	100 lbs.	42	29	Severe
Calcium cyanamid dust	200 lbs.	15	13	Severe
Sodium-calcium cyanamid dust	100 lbs. each	6	6	Severe
Check		14	14	Severe



Table 23. Comparison of the effectiveness of two chemical treatments for eradication of the aecial stage of mint rust and their effect on subsequent rust control.

Treatment	Rate per acre	Repli- cation	Number shoots infected:		Pairs of leaves dropped* and means ( $\bar{x}$ )	Disease index* and means ( $\bar{x}$ )
			Before treat- ment	10 days after treat- ment		
Potassium cyanate plus dinitro	10 lbs. plus $1\frac{1}{2}$ qts. in 100 gal. water	1	8	0	5.50	16.67
		2	5	0	5.00	16.67
		3	13	1	5.30	20.00
$\bar{x} = 5.27$					$\bar{x} = 17.85$	
Dinitro plus diesel oil	$1\frac{1}{2}$ qts. plus 20 gal. in 100 gal. water	1	13	3	4.35	18.33
		2	11	0	5.15	21.67
		3	7	0	4.85	17.78
$\bar{x} = 4.78$					$\bar{x} = 19.26$	
Untreated control		1	12	12	8.10	23.89
		2	5	5	9.15	28.33
		3	14	13	9.05	25.00
$\bar{x} = 8.77$					$\bar{x} = 25.74$	

\*Least significant difference at .05 for leaf drop means = 1.39; for disease index means = 4.66.

Table 24. Effect of chemical burn back on the number of mint shoots per square yard six weeks after treatment.

Treatment	Number of shoots per square yard in replication:			Mean no. shoots per sq. yard
	1	2	3	
Potassium cyanate plus dinitro in water	186	114	134	145
Dinitro plus diesel oil in water	158	131	161	150
Untreated control	108	93	88	96

An analysis of variance of leaf drop demonstrated that both treatments were significantly different from the controls at the 5 per cent level, but not different from each other. Similarly, the disease indexes of both treatments were significantly different from the controls, but not from each other.

The number of shoots per unit area (Table 24) appeared to be larger on the treated than on the control plots. However, the apparent increase in number of shoots is not significant at the 5 per cent level of probability. The fact that as many shoots were present on the treated as on the untreated plots can be explained on the following

basis. After "burning back" with chemicals, new shoots grow from buds in the leaf axils near the soil surface. In mints the leaves are opposite, hence, in some cases 2 new shoots grew in place of the one which was "burned back". In other cases shoots were killed by the treatments.

### Protectant Fungicides

#### Fungicide screening trials

During the growing season of 1951 an experiment was conducted to test the effectiveness of several widely used fungicides for the control of the urediospore stage of mint rust. A randomized block design with 5 replications and plots 10 feet square was employed. All applications were made with a hand sprayer except in the one case where Ferbam dust was used, it was applied with a hand duster. Four applications were made at 10 to 14 day intervals from the appearance of the first urediospores until three weeks before harvest. Rates of application were those recommended by the manufacturer for control of various other diseases.

Plots were individually cut, weighed and distilled. Each plot was sampled by cutting 12 plants selected at random, and rating the leaves at 3 positions on the main

stem for rust severity. The 3 positions at which leaves were numerically rated into rust classes were at the base, middle and top of the plant. The exact pair of leaves to be rated was determined by rating the first pair of basal leaves; counting 4 nodes and then rating the fifth pair of leaves, counting 4 more nodes and rating the tenth pair of leaves or the pair immediately below the terminal flower spike, whichever occurred first. A disease index for each plot was computed by applying a variation of the McKinney infection-index formula described on p. 57.

The treatments, rates of application and disease index in replication are given in Table 25. An analysis of variance of the disease index data in Table 25 resulted in a significant F-value for treatments. Further statistical tests (L.S.D. and extreme mean) revealed that Phygon reduced disease severity. All other treatments were not effective in reducing disease severity.

Yields of oil from each plot are given in Table 26. Statistical treatment of oil yield data resulted in a significant F-value for treatments. L.S.D. and extreme mean tests revealed that Phygon increased oil yields. None of the other treatments increased oil yields significantly.

The yield data in Table 26 were used to test the reliability of the disease index as previously described on

Table 25. Effectiveness of 10 materials for rust control as shown by a computed disease index.

Materials and rates	Disease index in replication:					Mean disease index*
	1	2	3	4	5	
Phygon 2,3-dichloro-1,4-naphthoquinone (50%) 1 lb. per 100 gal.	6.02	7.87	9.26	16.20	12.50	10.37**
Cop-o-zinc basic copper (42%) and zinc (11%) sulfates 4 lbs. per 100 gal.	16.20	18.33	15.74	31.94	25.00	21.44
Cuprocide cuprous oxide (90%) 1½ lbs. per 100 gal.	25.54	16.20	28.70	21.76	23.15	22.87
Captan n-trichloromethylthiotetra- hydrophthalimide (50%) 2 lbs. per 100 gal.	19.44	26.85	21.76	24.54	37.50	26.02
Copper-A tetra copper calcium oxychloride (45%) 4 lbs. per 100 gal.	26.39	25.92	30.55	28.70	26.39	27.59
Ferbamspray ferricdimethyldithiocarbamate (76%) 2 lbs. per 100 gal.	28.70	27.31	25.00	24.07	33.80	27.78



Table 25. (continued).

Materials and rates	Disease index in replication:					Mean disease index
	1	2	3	4	5	
Crag 341-C glyoxalidine acetates (50%) 1 pint per 100 gal.	25.00	22.68	32.01	24.07	37.96	28.15
Orthorix calcium polysulfides (26%) polyethylene glycol monoiso octylphenyl ether (10%) 2 qts. per 100 gal.	18.98	32.41	34.72	30.09	30.09	29.26
Lime sulfur calcium polysulfides (29%) 2 qts. per 100 gal.	21.30	29.63	40.74	26.85	34.72	30.65
Ferbam dust ferricdimethyldithiocarbamate (10%) 55 lbs. per acre	27.78	33.80	37.96	30.09	25.92	31.11
None (control)	19.44	36.11	35.65	37.50	41.20	33.98

\* Least significant difference at .01 = 8.71

\*\* Significantly different from check at .01



Table 26. Effectiveness of 10 materials for rust control as shown by yield of oil.

Material	Grams of oil in replication:					Mean oil yield in grams*
	1	2	3	4	5	
Phygon	64	67	76	59	67	66.6**
Captan	53	57	55	46	57	53.6
Cop-o-zinc	61	49	52	48	57	53.4
Cuprocide	50	58	44	49	64	53.0
Copper-A	55	46	58	51	53	52.6
Ferbam (spray)	47	49	60	52	47	51.0
Crag 341-C	50	46	53	55	44	49.6
Lime sulfur	45	43	52	51	51	48.4
Orthorix	47	53	42	39	60	48.2
Ferbam (dust)	44	41	50	53	48	47.2
None (control)	51	47	44	50	54	49.2

\* Least significant difference at .01 = 9.52.

\*\*Significant by L.S.D. at .01.

p. 62. Table 27 shows a comparison of yield and disease index for the various treatments. The data in Table 27 show that yield decreased as disease index increased; and both yield and disease index data show Phygon to be the fungicide exhibiting the best rust control possibilities.

Table 27. Comparison of disease index and oil yield means from peppermint plots treated with 10 fungicides.

Fungicides used	Mean disease index	Mean oil yield in grams
Phygon	10.37	66.6
Cop-o-zinc	21.44	53.4
Cuprocide	22.87	53.0
Captan	26.02	53.6
Copper-A	27.59	52.6
Ferbam (spray)	27.78	51.0
Crag 341-C	28.15	49.6
Orthorix	29.26	48.2
Lime sulfur	30.65	48.4
Ferbam (dust)	31.11	47.2
None (control)	33.98	49.2

Flavor evaluation of peppermint oil from plots treated with fungicides

In 1949 Steenland (26) conducted trials with ferbam (ferriodimethyldithiocarbamate) for mint rust control and found that the resulting oil after distillation contained up to 400 parts per million of carbon disulfide. Further tests were conducted which established that oil from mint treated with dithiocarbamates contained carbon disulfide

in quantities sufficient to make the oil undesirable.

Other fungicides used on mint might also cause undesirable residues or flavors in the oil. Samples of oil from plots treated with the fungicides listed in Table 25 were submitted to the Beech-Nut Packing Company for flavor analyses. Dr. L. G. Cox of the Beech-Nut Packing Company stated that on the basis of their organoleptic panel tests the oil from the various treatments were placed in the following order of preference:

- |               |              |   |             |
|---------------|--------------|---|-------------|
| 1. Phygon     | Captan       | ) |             |
| 2. Crag 341-G | Lime Sulfur  | ) |             |
| 3. Copper-A   | Ferbam spray | ) | undesirable |
| 4. Cuprocide  | Ferbam dust  | ) |             |
| 5. Orthorix   |              |   |             |
| 6. Cop-o-zinc |              |   |             |

#### Fungicidal sprays and dusts

Since Phygon was the only fungicide that gave good rust control and was also selected as the best material by oil flavor evaluation, it was the only fungicide chosen for further rust control trials.

During the summer of 1953, field trials were conducted to determine: 1) whether Phygon sprays or dusts were most effective for rust control; 2) what concentrations of Phygon dusts or sprays were most effective; 3) how many spray or dust applications were necessary for rust control;

and 4) whether mint treated with Phygon sprays or dusts on a field scale would have an undesirable flavor when distilled by standard commercial methods. The experimental design consisted of a randomized block with 4 replications of  $\frac{1}{2}$  acre plots.

Dusting equipment consisted of a trailer-mounted, tapered-boom duster made by the Tufts Company, Alhambra, California. The duster in operation is shown in Figure 10. Spray equipment consisted of a tractor-mounted spray boom fitted with cone-type spray nozzles. A push-bar parallel with and ahead of the spray boom was employed to tip the mint plants forward ahead of the spray pattern, thus exposing more of the undersides of the leaves to the spray. The spray equipment in operation is shown in Figure 11.

Dust and spray applications were made at 13 to 17 day intervals beginning May 29. Either 3 or 5 applications were made. Sprays were applied at Phygon concentrations of  $\frac{1}{4}$ ,  $\frac{1}{2}$  and 1 pound per 100 gallons and approximately 100 gallons per acre. Phygon dust applications were  $1\frac{1}{4}$ ,  $2\frac{1}{2}$  and 5 per cent dusts at 40 pounds per acre.

Just prior to harvest all plots were sampled by selecting 10 plants at random and rating them for rust severity by the method previously described. From the rust



Figure 10. Duster used in fungicidal control trials.



Figure 11. Sprayer used in fungicidal control trials.



severity ratings a disease index was computed for each plot. Plots in each treatment were distilled separately and oil samples collected for flavor evaluation.

The mean disease index from both dust and spray plots is given in Table 28. Analysis of variance of spray treatments revealed no significance for treatments, therefore, sprays were not effective in reducing rust severity. Analysis of variance of dust treatments revealed a significant F-value for treatments. L.S.D. tests among dust treatment means revealed that both  $2\frac{1}{2}$  and 5% dusts with 5 applications reduced rust severity at the one per cent level of probability. Three dust applications did not reduce rust severity at any of the rates used.

Oil samples representing all treatments were submitted to the A. M. Todd Company, Kalamazoo, Mich.; the Beech-Nut Packing Company, Canajoharie, New York; and the Wm. Wrigley Jr. Company, Chicago, Ill. for flavor evaluation. All three companies found no consistent differences in flavor of oil from treated and untreated plots. Oil from treated plots was judged as good as oil from untreated control plots.

#### Resistant Varieties

Breeding and selection of resistant varieties is one of the most successful methods of controlling rust

Table 28. Effectiveness of Phygon sprays and dusts at different rates and times of application for mint rust control as shown by a computed disease index.

Treatment and rate	No. of applications	Mean disease index
$\frac{1}{4}$ lb.	3	34.62
	5	35.60
spray $\frac{1}{2}$ lb.	3	36.11
	5	34.44
1 lb.	3	33.33
	5	31.50
None	-	35.55
$1\frac{1}{4}\%$	3	32.36
	5	30.55
Dust $2\frac{1}{2}\%$	3	25.69
	5	19.78**
5%	3	26.25
	5	20.11**
None	-	33.33

\*\*L.S.D. at .01 for dust treatment means = 7.68

diseases of plants. Plant breeding programs for the development of peppermint and spearmint varieties resistant to the destructive Verticillium wilt disease are now being conducted by the United States Department of Agriculture in cooperation with Purdue University, by Michigan

State College and by the A. M. Todd Company, Kalamazoo, Michigan. Breeding of rust resistant spearmint varieties is also being conducted by Michigan State College and the A. M. Todd Company.

Mentha crispa L. has been used as the wilt resistant parent in crosses with M. piperita by Nelson (16) and by E. C. Stevenson and C. A. Thomas\* of the U. S. Department of Agriculture. Since 1951 hybrids of Mentha crispa x Mentha piperita have been made available to the author through the courtesy of Dr. C. A. Thomas. These hybrids have been tested in the greenhouse for resistance to the race of Puccinia menthae attacking peppermint in Oregon.

Test plants were grown either from rhizomes or rooted cuttings in 6-inch clay pots and inoculated by spraying a urediospore suspension onto the undersides of the leaves. At least three replications of each variety were employed for each test. After inoculation high humidity was maintained around the plants for 48 hours by frequently spraying water on a heavy muslin cover placed over the greenhouse bench. Three weeks after inoculation and again one week later all plants were examined and rated for rust resistance.

---

\*Personal communication with Dr. C. A. Thomas

Five different types of reaction to rust were consistently observed among the hybrids tested. The different uredial reactions were assigned numerical values characterized as follows:

- 0 = no evidence of infection.
- 1 = infection followed by the appearance of small necrotic spots; no sporulation.
- 2 = infection followed by necrotic spots; sporulating sori minute.
- 3 = infection followed by necrosis or chlorosis; sporulating sori small.
- 4 = infection followed by abundant, large, sporulating sori.

Ninety-one hybrids and 6 species of Mentha have been tested for their reaction to the race of Puccinia menthae designated as race 2 by Baxter and Cummins (4). The uredial reactions of the six mint species listed in Table 29 agree with those found by Baxter and Cummins for race 2. Each of 91 M. crispa x M. piperita hybrids were tested and 4 were found to be immune, 31 highly resistant, 14 moderately resistant, 16 moderately susceptible and 26 susceptible to race 2 of P. menthae. Other races of P. menthae capable of attacking the immune and resistant hybrids may exist in Oregon.

Hybrids with different uredial reactions to race 2 could be used as differential hosts to detect other races

Table 29. Resistance of 5 species and 91 hybrids of Mentha to race 2 of Puccinia menthae.

Species or hybrid	Number of plants tested	Number of plants showing rust reaction:				
		0	1	2	3	4
<u>Mentha</u> <u>crispa</u> L.	3	3				
" <u>piperita</u> L.	3					3
" <u>spicata</u> L.	3	3				
" <u>cardiaca</u> L. (Scotch)	3				3	
" <u>pulegium</u> L.	3	3				
" <u>canadensis</u> L.	3					3
91 hybrids of <u>Mentha</u> <u>crispa</u> x <u>M. piperita</u>	273 <sup>a</sup>	12 <sup>b</sup>	93 <sup>c</sup>	42 <sup>d</sup>	48 <sup>e</sup>	78 <sup>f</sup>

a	3	plants	of	each	hybrid	were	tested
b	3	plants	each	of	4	hybrids	
c	3	"	"	"	31	"	
d	3	"	"	"	14	"	
e	3	"	"	"	16	"	
f	3	"	"	"	26	"	

of Puccinia menthae which might be present in Oregon. Collections of mint rust aeciospores were made from 3 mint species in 12 different localities in the state. Aeciospores rather than urediospores were used to detect races because Stakman and Loegering (24) and 25) demonstrated that different races of wheat stem rust were 4 to 20 times more prevalent in aeciospore collections.

Mentha piperita, Mentha spicata and five M. crispa x M. piperita hybrids showing rust reactions of 0, 1, 2, 3 and 4 respectively, were inoculated with each of 12



collections of aeciospores. Rust reactions were determined 4 weeks after inoculation and compared with the known reaction of the test plants to race 2. Table 30 shows that two races of rust were differentiated by the reactions of the test plants. In this test one race always originated from either M. piperita or M. canadensis and caused a reaction identical with race 2 on all test plants. The other race always originated from M. spicata and was not infective to any of the test plants except M. spicata. It appears that only one race of Puccinia menthae capable of attacking peppermint is prevalent in Oregon at the present time.



Table 30. Rust reaction produced on 2 species and 5 hybrids of Mentha by aeciospores collected from 3 mint species in 12 localities.

Locality number	Rust reaction on:						
	<u>M.</u> <u>piperita</u>	<u>M.</u> <u>spicata</u>	Hybrids with reaction to race 2 of:				
			0	1	2	3	4
1 <u>Mentha piperita</u>	4	0	0	1	2	3	4
2       "      "	4	0	0	1	2	3	4
3       "      "	4	0	0	1	2	3	4
4       "      "	4	0	0	1	2	3	4
5       "      "	4	0	0	1	2	3	4
6 <u>Mentha canadensis</u>	4	0	0	1	2	3	4
7       "      "	4	0	0	1	2	3	4
8 <u>Mentha spicata</u>	0	4	0	0	0	0	0
9       "      "	0	4	0	0	0	0	0
10       "      "	0	4	0	0	0	0	0
11 <u>Mentha piperita</u>	4	0	0	1	2	3	4
12       "      "	4	0	0	1	2	3	4

## SUMMARY AND CONCLUSIONS

Peppermint rust caused by Puccinia menthae overwinters as teliospores on fallen leaves, mint stubble and rhizomes, and on the soil surface. Teliospores are produced from May to December, but heaviest production occurs during October and November. Maximum germination of teliospores produced in November occurred during December and January, but spores were still germinating in February, 108 days after being produced.

Infection of young mint shoots by basidiospores begins as early as December and continues through March. Low temperature retards the development of spermogonia and aecia. An average of 10 days were required for mature aecia to develop from basidiospore infections at 20° C., whereas, 27 days were required at 10° C.

Aeciospores are produced from February to June, but development of urediospores from aeciospore infections during February and March is greatly retarded by low temperatures. Infections during February and early March required an incubation period of approximately 6 weeks under field conditions, while under greenhouse conditions favorable for rust development the incubation period was 2 weeks. Aeciospores rapidly lost their viability at temperatures below 0° C. and above 30° C., while viability

was retained longest at 5° C.

Initial spread of rust by aeciospores is limited to a few feet.

No evidence was found that urediospores overwinter in the field under Oregon conditions. The time interval between the last viable urediospores found in the fall and their appearance the succeeding season was 105 days.

Rust mycelium and infected host cells were killed before rust sporulation at a temperature of 100° C. for four hours while after rust sporulation they were killed at 90° C. for four hours. Urediospores produced in sori exposed to direct solar radiation lost their viability much more rapidly than urediospores protected from direct solar radiation.

Crop losses caused by Puccinia menthae occur during all stages of development of the parasite. Most losses caused by basidiospore infection, spermogonia and aecia come about from invasion of the infected area by secondary organisms and subsequent death of the infected shoot or rhizome.

Crop losses caused by urediospore infections include leaf drop, inhibition of lateral shoot development and destruction of oil glands by erupting sori.

Plants with heavy urediospore infection produce rhizomes smaller in diameter, length and weight than healthy

plants.

A method of evaluating rust severity and control data based on percentage destruction of oil glands on the leaves proved to be a valuable tool for evaluating the effectiveness of fungicides.

Control measures of thorough plowing to cover all old stubble and plant debris, eradication of wild and escaped mint near commercial plantings, eradication of mint infected with the aecial stage within plantings by contact plant-killer chemicals and properly timed irrigation all effectively reduced rust losses.

Of 9 protectant fungicides tested only Phygon controlled rust effectively.

Phygon dusts were more effective than sprays; and 2½% dust was more effective than 1¼% dust, and equally as effective as 5% dust.

Two races of Puccinia menthae were found in Oregon. One of these races attacks Mentha piperita, M. canadensis, and certain M. crispa x M. piperita hybrids, while the other race attacks M. spicata. The two races are not cross infective on any of the hosts mentioned above.

Of 91 hybrids of M. crispa x M. piperita tested for rust resistance, 4 were immune, 31 highly resistant, 14

moderately resistant, 16 moderately susceptible and 26 susceptible to the race of Puccinia menthae attacking commercial peppermint.



## BIBLIOGRAPHY

1. Arthur, Joseph C. The plant rusts. New York, Wiley, 1929. 446p.
2. \_\_\_\_\_ Manual of the rusts in United States and Canada. Lafayette, Indiana, Purdue research foundation, 1934. 438p.
3. Baxter, John W. The occurrence of rust on peppermint in Western Ontario. U. S. Dept. of Agriculture, Plant Disease Reporter 35:418. 1951.
4. \_\_\_\_\_ and George B. Cummins. Physiologic specialization in Puccinia menthae Pers. and notes on epiphytology. Phytopathology 43:178-180. 1953.
5. Bullis, D. E., F. E. Price and Dale E. Kirk. Relationship of maturing and weathering to yield and quality of peppermint oil. Corvallis, Oregon state college, 1948. 15p. (Oregon. Agricultural experiment station. Bulletin 458)
6. Cruchet, Paul. Contributions a l'etude biologique de quelques Puccinies sur Labiees. Centralblatt fur bacteriologie, parasitenkunde und infektion-skrankheiten 17:212-224. 1907.
7. Dietz, S. M., A. P. Steenland and C. E. Horner. Mint rust (Puccinia menthae) epiphytotic in the Northwest. Phytopathology 41:938. 1951.
8. Dimock, A. W. and Kenneth F. Baker. Effect of climate on disease development, injuriousness, and fungicidal control as exemplified by snapdragon rust. Phytopathology 41:536-552. 1951.
9. Drummond-Goncalves, R. A. Ferrugem da hortela pimenta. Biologico 9:383-386. 1943.
10. Hopp, Henry. Control of atmospheric humidity in culture studies. Botanical gazette 98:25-44. 1936.
11. Horner, Chester E. Root and rhizome rot of peppermint. Phytopathology 42:514-515. 1952.



12. Horsfall, J. G. and J. W. Heuberger. Measuring magnitude of a defoliation disease of tomatoes. *Phytopathology* 32:226-232. 1942.
13. Marsh, R. W., H. Martin and R. G. Munson. Studies upon the copper fungicides. 3. The distribution of fungicidal properties among certain copper compounds. *Annals of applied biology* 24:853-866. 1937.
14. Muhle, E. Zur biologie und bekampfung des pfefferminz-rostes, Puccinia menthae Pers. *Die Pharmazie* 10:541-546. 1951.
15. McKinney, H. H. Influence of soil temperature and moisture on infection of wheat seedlings by Helminthosporium sativum. *Journal of agricultural research* 26:195-218. 1923.
16. Nelson, Ray. Verticillium wilt of peppermint. East Lansing, Michigan state college, 1950. 259p. (Michigan. Agricultural experiment station. Technical bulletin 221)
17. \_\_\_\_\_ Mint rust and its control. Michigan state college agricultural experiment station quarterly bulletin 34:436-455. 1952.
18. Niederhauser, J. S. The rust of greenhouse-grown spearmint and its control. Ithaca, Cornell University, 1945. 30p. (Cornell university. Agricultural experiment station. Memoir 263)
19. Owen, Herbert Elmer Jr. Certain factors affecting the establishment of the douglas fir (Pseudotsuga taxifolia (Lamb.) Britt.) seedlings. Master's thesis. Corvallis, Oregon state college, 1953. 71 numb. leaves.
20. Peltier, George L. A study of the environmental conditions influencing the development of stem rust in the absence of an alternate host. Lincoln, Nebraska, University of Nebraska, College of agriculture, 1923. 52p. (Nebraska. Agricultural experiment station. Research bulletin 25)
21. Persoon, Christiaan Hendrik. Puccinia menthae. *Synopsis methodica fungorum* p227. 1801.

22. Raines, M. A. Vegetative vigor of the host as a factor influencing susceptibility and resistance to certain rust diseases of the higher plants. American journal of botany 9:183-203. 1922.
23. Sievers, A. F. and E. C. Stevenson. Mint farming. Washington, U. S. Government printing office, 1948. 30p. (U. S. Dept. of agriculture. Farmers bulletin no. 1988)
24. Stakman, E. C. and W. Q. Loegering. Physiologic races of *Puccinia graminis* in the United States in 1950. Washington, D. C., U. S. Dept. agriculture, bureau of plant industry, soils, and agricultural engineering, 1951. 16p. (Unnumbered publications issued July, 1951. Mimeographed)
25. \_\_\_\_\_ Physiologic races of *Puccinia graminis* in the United States 1945-1949. Washington D. C., U. S. Dept. agriculture, bureau of plant industry, soils, and agricultural engineering, 1950, 19p. (Unnumbered publications issued November, 1950. Mimeographed)
26. Steenland, A. P. The peppermint rust situation in Oregon. Proceedings of the Oregon essential oil growers league, second annual meeting, 1951. Corvallis, Oregon state college, 1951. 37p. (Mimeographed)
27. Vergovsky, V. I. Mint rust and methods of its control. Simferpol (Crimea) All union scientific research institute for medicinal and medicinal-technical plants. Bulletin 3:5-54. 1935. (Translation from the Russian by John S. Niederhauser, 1943, deposited in Cornell University plant pathology library)