

structed in this laboratory for further studies of this unusual reaction.

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Studies on the Host Range of the *Verticillium* that Causes Wilt of *Mentha piperita* L.

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Peppermint, *Mentha piperita* L., is one of the important crops in southern Michigan and northern Indiana, where it is cultivated exclusively for its essential oils. The muck soils of these states are particularly well adapted to the cultivation of this crop, and in those areas such soils are devoted almost entirely to peppermint oil production. However, during the past decade there has been a rapid decline in peppermint acreage, particularly in Michigan, as a result of infestation of the soil with a fungus which causes the serious mint disease known as *Verticillium* wilt.

This disease was first reported in Michigan by Nelson (1) in 1926, and since that time it has become common throughout the peppermint-raising areas in that state. In 1941 it was noted by Baines (2) for the first time in Indiana, where it has likewise become very prevalent and important. Baines identified the causal agent as *Verticillium albo-atrum* R & B, but Nelson (3), in 1947, subsequently claimed that it more nearly resembles *V. dahliae*, a species which Rudolph (4) had previously believed to be invalid.

So far no completely successful control measures have been developed. Breeding of resistant varieties has been attempted, but this work is greatly hampered by the fact that the flowers of *M. piperita* are male sterile. At the present time crop rotation appears to be the most promising means of control. The success of such measures, however, will depend on the susceptibility of other crop and weed plants to the disease, the ability of the causal agent to persist for long periods of time as a saprophyte in the soil or in plant debris, the agricultural practices adopted, and on many other factors.

In view of the economic importance of *Verticillium* wilt and its rapid spread in Michigan and Indiana, extensive research programs have been initiated on the identity, variability, and pathogenicity of the causal organism, control measures, susceptibility, and host range. Nelson (3) reported that the isolate of *V. albo-atrum* from *M. piperita* is specific to this host and closely related species of *Mentha*. He also stated that *M. piperita* is resistant to isolates of *V. albo-atrum* from a number of unrelated host plants.

The present study was undertaken to ascertain the susceptibility of the major weed and crop plants of the muck soil regions of northern Indiana, to determine the ability of *V. albo-atrum* to exist as a saprophyte in the soil and on vegetable debris, and to check the above reports on the host specificity of the causal agent and the resistance of *M. piperita* to isolates from unrelated hosts.

In the susceptibility tests the following weed plants and their seeds were collected from within and around the fields in which *M. piperita* is cultivated and were used as host plants under greenhouse conditions:

Lactuca scariola L.
Mentha rotundifolia L.
Echinochloa crus-galli Beauv.
Erysimum cheiranthoides L.
Chenopodium album L.
Cannabis sativa L.
Lepidium virginicum L.
Bidens vulgata L.
Taraxacum vulgare Lam.
Persicaria hydropiper L.
Amaranthus retroflexus L.
Portulaca oleracea L.
Oenothera biennis L.
Aster ericoides L.
Ambrosia trifida L.
Rumex altissimus L.
Plantago major L.
Rudbeckia hirta L.
Urtica dioica L.
Erigeron canadensis L.
Physalis subglabrata M & B

As soon as these plants had attained appropriate size they were inoculated with pure cultures of causal agent via the soil, and after 10 days a second inoculation was made in the same manner. Adequate controls were set up for each species. The fungus isolate used in these experiments was taken from *M. piperita* and will be referred to as *V. albo-atrum* B11B to distinguish it from isolates from other host plants. In the course of a period of 45 days after inoculation, attempts were made to recover the causal organism from the roots and stem of each inoculated plant, using techniques that have proved successful in isolating *V. albo-atrum* B11B from *M. piperita*.

The results of these susceptibility and specificity tests were striking. *V. albo-atrum* was isolated from only one of the tested species, *M. rotundifolia*. This species is closely related to *M. piperita*, which may possibly be the reason for its susceptibility. In addition to the above greenhouse and laboratory tests, field surveys were conducted during the late spring, summer, and fall seasons. Weed plants were carefully observed within and along the boundaries of fields of *M. piperita*. No definite symptoms of the disease were found. Nevertheless, specimens were collected on each survey, and attempts were made to isolate *V. albo-atrum* B11B from their roots and stems. None of these plants yielded the causal agent.

Economic plants were selected as representative of the various types of crop plants cultivated in the muck soil regions of northern Indiana. Some of the species of crop plants utilized are of somewhat minor

importance but, as the trend toward diversification continues, may become more significant. The species of plants used in these experiments were:

Solanum tuberosum var. Chippewa
Lycopersicon esculentum var. John Baer
L. esculentum var. Southland
Solanum melongena var. Black Beauty
Capsicum annuum var. Calwonder
Brassica oleracea var. Golden Aeres
Cucumis sativus var. David Bland
Allium cepa var. Yellow Globe
Zea mays saccharata

These plants were inoculated either by dipping the root system of the young seedlings in a heavy suspension of spores, sclerotia, and mycelia of *V. albo-atrum* and transplanting, or by working pure cultures of this fungus into the soil around the base of the plants. Control plants were maintained, and all the plants were allowed to grow for a period of 45 days. At the end of this period, attempts were made to recover the causal agent from all the plants inoculated.

V. albo-atrum B11B was recovered from two species of plants inoculated—namely, from the roots and aerial stems of 6 of 20 plants of *Solanum melongena* var. Black Beauty—and also from 4 of 20 plants of *Capsicum annuum* var. Calwonder, but only from the primary and lateral roots and never from the aerial stem.

These results seem to indicate that the isolate of *V. albo-atrum* from *M. piperita* is not as specific to that genus as has been believed.

The ability of *V. albo-atrum* B11B to exist as a saprophyte was determined by inoculating sterilized plant debris with pure cultures of the fungus. At the end of an incubation period of 30 days the relative amount of mycelial development was used as the criterion of the suitability of each substrate as a growth medium. The results of this experiment are shown in Table 1. It is to be noted that growth was excellent on *M. piperita* and *Chenopodium album*, and good on all other species except *Cannabis sativa*.

TABLE 1

Plant debris	Amt. growth
<i>Solanum tuberosum</i> L.	**
<i>Lycopersicon esculentum</i> Mill.	**
<i>Capsicum annuum</i> L.	**
<i>Brassica oleracea</i> L.	**
<i>Mentha piperita</i> L.	***
<i>Amaranthus retroflexus</i> L.	**
<i>Chenopodium album</i> L.	***
<i>Portulaca oleracea</i> L.	**
<i>Cannabis sativa</i> L.	*
<i>Oenothera biennis</i> L.	**

*** Excellent.
 ** Good.
 * Poor.

The fact that the debris of *M. piperita* proved to be an excellent substrate is highly significant from the standpoint of control. After the oil has been distilled from the leaves and stems, the debris is used by many growers as organic fertilizer, and doubtless serves as

a medium for propagating the fungus in the soil.

The final phase of this investigation dealt with the susceptibility of *M. piperita* to isolates of *V. albo-atrum* from other plants. Healthy plants of *M. piperita* were inoculated (via the soil, as in the previous experiments) with pure cultures of *V. albo-atrum* from potato, tomato, eggplant, nightshade, okra, honeydew melon, radish, boysenberry, and *Aralia japonica*. The results of these tests were negative except in two cases. *V. albo-atrum* No. 119 from tomato proved to be cross-infective with *M. piperita*, as did the isolate No. 53 from radish. In the latter case, however, the fungus was recovered only from the stem below the soil level and the smaller lateral roots of *M. piperita*.

From the standpoint of establishing a feasible crop rotation program to control peppermint wilt, our results seem to indicate that the natural flora and other economic plants of the muck soil regions of northern Indiana are relatively insignificant as hosts of *V. albo-atrum* B11B. Furthermore, our results show that this isolate is an efficient saprophyte and, barring inhibition by other microorganisms and environmental factors, it may persist on plant debris and in the soil for long periods of time. In addition, the results obtained indicate that *V. albo-atrum* B11B is not as specific in host range as previously believed. These results also support evidence from other sources for the existence of strains of *V. albo-atrum* which vary in host specificity and pathogenicity. Research now in progress is being done in an attempt to determine the stability of the proposed strains of the fungus *V. albo-atrum*.

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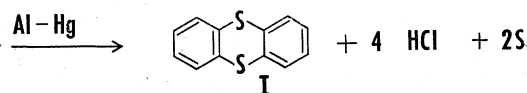
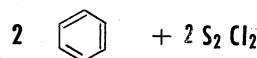
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Formation of Thianthrene by a Free Radical Mechanism

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Cohen and Skirrow (1) observed the formation of thianthrene (I) from benzene and sulfur monochloride



in the presence of an aluminum amalgam. These authors found that the reaction was very rapid and that the aluminum-mercury couple, used in small amount, was not affected by the reactants. These data seemed