OBSERVATIONS ON THE ORIGIN AND NATURE OF VERDICILLIUM DAHLIAE COLONIZING PLANT ROOTS

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Abstract

In naturally infested field soils V. dahliae colonized the roots of a wide range of plants and some species consistently yielded more colonies per unit length of root than others. However, susceptible species were colonized no more frequently than species immune to systemic infection. Discrete colonies, about 2·0 mm in length, were found to occur at random along the root system. Thus each colony on a section of root appears to originate from a different microsclerotium. On seedling plants the size of the colonies was fairly uniform, irrespective of how abundantly the roots were colonized, which suggested that factors influencing germination of microsclerotia are independent of factors affecting growth at the root surface. Most of the colonies were removed when exposed to low concentrations (2–64 p.p.m.) of mercury for 1 min, although the colonies on three susceptible species were less vulnerable to the fungicide than the colonies on one immune species. These observations suggest that the colonies are mostly restricted to the rhizoplane or to superficial infection sites in the root cortex.

I. INTRODUCTION

Verticillium dahliae Klebahn can colonize the roots of a wide range of plants including species which are immune to systemic infection (Martinson 1964; Lacy and Horner 1966; Harrison and Isaac 1969; Evans 1971). Martinson reported that the fungus formed microsclerotia in the roots of many immune species† and suggested that these could maintain the fungus in the absence of recognized hosts, but this has not been confirmed. Evans (1971) found that the fungus could be isolated only infrequently from dead roots of plants which were not systemically infected. Lacy and Horner (1966) reported that soil populations of V. dahliae increased more in the rhizosphere of susceptible species and that these were colonized more frequently than immune species. However, Evans (1971) believed that immune species may be just as prone to colonization as susceptible species. For research aimed at reducing the level of inoculum in soil the question of whether V. dahliae can maintain itself on immune species is obviously an important one and needs to be answered.

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† The term immune species is used to describe plants which are not systemically infected in the particular environment in which they are grown. It is recognized, however, that a species may be immune to systemic infection in one area and yet quite susceptible in another where different strains of the fungus exist.

In 1969, at the Agricultural Research Station at Tamworth, a project was initiated to study the influence of immune species on the survival of *V. dahliae* in cotton field soils of the Namoi Valley. This paper reports on a part of this work dealing with the origin, nature, and extent of root infection in a number of plants. The effect of low concentrations of fungicidal materials on these colonies is also reported.

II. Materials and Methods

(a) Verticillium-infested Soil

The soil used in these studies was from a naturally infested cotton field in the Namoi Valley of New South Wales. It was one of the grey and brown soils of heavy texture, about pH 8.0, which predominate in the cotton-growing area. The soil was collected in the winter of 1969 after the trash from the previous cotton crop had been ploughed under. The soil was dried, sieved through a mesh screen (0.5 cm), and stored until required. Samples of the soil assayed by the method of Evans *et al.* (1967) gave population estimates ranging from 60 to 150 microsclerotia per gram air-dry soil. For each experiment the required amount of soil was thoroughly mixed to ensure a random distribution of inoculum throughout the sample.

(b) Verticillium-free Soil

In some of the experiments it was necessary to reduce the level of inoculum in the infested soil. This was done by mixing it with a soil of the same type which was apparently free from *V. dahliae*. The *Verticillium*-free soil was also collected in the winter months after the trash from a cotton crop had been ploughed under. *Verticillium* wilt had never been observed in cotton growing in this field and attempts to trap the fungus on plant roots were not successful. This soil was sieved and stored similarly to the infested soil.

(c) Growing Plants in the Glasshouse

Except where otherwise stated, plants were grown in the infested soil in 15-cm diameter clay pots without the addition of fertilizer. Pots of soil sown with seeds of the test plants were kept in an air-conditioned glasshouse at 25.0 ± 2.0°C during the day and 15.0 ± 2.0°C during the night. After emergence the seedlings were thinned to two to six plants per pot, depending on the size and vigour of the particular species, and watered as often as necessary to maintain adequate moisture for growth. In all experiments the plants were harvested 20 days after seedling emergence.

(d) Isolation of *V. dahliae* from Intact Roots

*V. dahliae* was isolated from segments of plant root on ethanol-streptomycin agar (Nadakavukaren and Horner 1959) containing 50 p.p.m. tetracycline (herein referred to as ethanol-streptomycin agar). Prior to plating, the roots were washed in running tap water, cut into segments 2 cm in length, except where stated otherwise, rinsed in sterile water, and blotted. These segments were incubated at room temperature for 7–10 days before examination under a dissecting microscope for *V. dahliae*.

(e) Isolation of *V. dahliae* from Fragmented Roots

Washed segments of root were prepared as before and fresh weights were recorded for samples of known lengths. These samples were fragmented in a Braun multi-speed blender for 2 min and a series of dilutions was prepared from the resulting suspensions. Five 1-ml samples were taken from each dilution and distributed over the surface of plates of ethanol-streptomycin agar which had been prepared 10–12 days earlier. The excess moisture was quickly absorbed by the agar, leaving the fragmented root tissue on the surface. After incubation for 10 days at room temperature the colonies of *V. dahliae* on these plates were recorded.
III. Experimental

(a) Colonization of Plant Roots by *V. dahliae*

The 23 species of weeds and cultivated plants from eight families from which colonies of *V. dahliae* were recorded are listed in the following tabulation:

**CHENOPODIACEAE**
- Chenopodium album L. (fat hen)

**COMPOSITAE**
- Carthamus tinctorius L. (safflower)
- Helianthus annuus L. (sunflower)
- Silybum marianum Gaertn. (variegated thistle)
- Xanthium pungens Wallr. (Noogoora burr)

**CONVOLVULACEAE**
- Ipomoea londochylylla J. M. Black (potato vine)

**GRAMINEAE**
- Hordeum vulgare L. (barley)
- Sorghum vulgare Pers. (grain sorghum)
- Triticum aestivum L. (wheat)
- Zea mays L. (maize)

**LABIATAE**
- Lamium amplexicaule L. (deadnettle)
- Marrubium vulgare L. (horehound)
- Salvia officinalis L. (common sage)
- Salvia verbenaca L. (wild sage)

**LEGUMINOSAE**
- Dolichos lablab L. (dolichos)
- Glycine max L. (soybean)
- Phaseolus aureus Roxb. (mung bean)
- Pisum arvense L. (field pea)

**MALVACEAE**
- Gossypium hirsutum L. (cotton)

**SOLANACEAE**
- Datura inoxia Mill. (downy thornapple)
- Datura stramonium L. (common thornapple)
- Lycopersicon esculentum Mill. (tomato)
- Solanum nigrum L. (nightshade)

*V. dahliae* was also recorded on five cultivars of soybean and eight cultivars of cotton, all of which varied in susceptibility to systemic infection.

In these experiments five pots of each species were harvested, 50 segments were cut from the roots in each pot and cultured for *V. dahliae*, and the number of colonies per unit length of root recorded. In another experiment the fresh weight of the root segments was recorded for five species and colonization was expressed both as number of colonies per gram fresh weight and number of colonies per 100 cm of root.

**Table 1**

<table>
<thead>
<tr>
<th>Plant</th>
<th>Host reaction</th>
<th>Colonies of <em>V. dahliae</em> per 100 cm</th>
<th>Colonies of <em>V. dahliae</em> per gram</th>
<th>Fresh weight per 100 cm root (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat cv. Festiguay</td>
<td>I</td>
<td>6±1·6†</td>
<td>117±32·1</td>
<td>0·05±0·00</td>
</tr>
<tr>
<td>Cotton cv. Deltapine</td>
<td>S</td>
<td>21±2·4</td>
<td>92±13·0</td>
<td>0·23±0·00</td>
</tr>
<tr>
<td>Smoothleaf</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soybean cv. Lee</td>
<td>I</td>
<td>25±2·6</td>
<td>137±18·5</td>
<td>0·20±0·02</td>
</tr>
<tr>
<td>Common thornapple</td>
<td>S</td>
<td>62±3·2</td>
<td>753±63·6</td>
<td>0·08±0·00</td>
</tr>
<tr>
<td>Noogoora burr</td>
<td>S</td>
<td>30±3·6</td>
<td>284±37·1</td>
<td>0·11±0·01</td>
</tr>
</tbody>
</table>

* S, susceptible; I, immune. † Standard error.

Some species consistently yielded more colonies per unit length of root than others (Fig. 1) but susceptible species were colonized no more frequently than immune species. When the population of *V. dahliae* on roots was expressed as colonies per gram fresh weight (Table 1), those species with fine roots of low fresh weight per unit length yielded relatively more colonies than species with coarser roots.
of high fresh weight per unit length. Cotton, for example, yielded significantly more colonies per unit length of root than wheat, but there was no difference between the number of colonies per gram weight of roots from the two species. Again, there was no evidence that the number of colonies per unit length was correlated with surface area of the roots. Common thornapple, for example, was consistently colonized more frequently than cotton and soybean with coarser roots of greater surface area per unit length.

KEY

1. Datura stramonium
2. Datura innoxia
3. Lycopersicon esculentum
4. Carthamus tinctorius
5. Xanthium pungens
6. Solanum nigrum
7. Phaseolus aureus
8. Gossypium hirsutum
9. Ipomoea lonchophylla
10. Pisum arvense
11. Dolichos lablab
12. Glycine max
13. Silybum marianum
14. Salvia verbenaca
15. Helianthus annuus
16. Salvia reflexa
17. Hordeum vulgare
18. Sorghum vulgare
19. Zea mays
20. Triticum aestivum
21. Marrubium vulgare
22. Chenopodium album
23. Lamium amplexicaule

Fig. 1.—Histogram showing the mean number of colonies of *V. dahliae* per 100 cm of root obtained from 23 species. □ Immune species. ■ Susceptible species.

In two experiments with cotton there was no evidence that the highly susceptible cultivars such as Deltapine Smoothleaf were more prone to colonization by *V. dahliae* than more resistant types such as Acala SJ1. The other cultivars (Auburn 56, Coker 100-W, Deltapine 45, Deltapine 5540, Hopicala, and Stoneville 7-02) all yielded the same number of colonies per unit length of root as Deltapine Smoothleaf and Acala SJ1. On the other hand, some soybean cultivars yielded more colonies per unit length than others, as indicated in the following tabulation:

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Hampton</th>
<th>Lee</th>
<th>Bragg</th>
<th>Ogden</th>
<th>Hardee</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colonies per 100 cm</td>
<td>17.7</td>
<td>20.4</td>
<td>23.3</td>
<td>24.2</td>
<td>27.2</td>
</tr>
</tbody>
</table>

(*b*) Origin of *V. dahliae* on Plant Roots

In two experiments the origin of the colonies of *V. dahliae* on plant roots was investigated. The first involved measuring the length of the colonies to see if hyphal growth could account for some species yielding more colonies per unit length of root than others. In the second experiment the distribution of the colonies on the roots
was studied. It was planned on the assumption that if microsclerotia were distributed at random in soil, and each propagule gave rise to just one colony on a section of root, the colonies themselves would be distributed at random. Conversely, if microsclerotia distributed at random in soil germinate, either directly or indirectly, to form more than one colony on a section of root, these colonies would likely be clustered, just as they would be if large colonies fragmented to form a number of smaller colonies.

(i) Measurement of Colony Length

Colonies of *V. dahliae* on plant roots are indistinguishable from other fungi and cannot be observed. Hyphal growth along roots was measured indirectly by counting the number of colonies on root segments of different length. Short (0·25 cm) segments invariably yielded a few more colonies per unit length of root than long (5·0 cm) segments, which indicated that some colonies were being cut. The difference between the two counts was used as an estimate of the number of divided colonies. Colony length was then calculated from $P = n/s/l$, where $P$ is the probability of dividing a colony with a single cut, $n$ is the number of colonies from a length of root $l$ (where $l$ is the maximum practical segment length), and $s$ is the mean length of the colonies. The ratio of the number of divided colonies to the total number of colonies from long segments provided an empirical estimate of $P$ for the calculation of $s$.

![Diagram](image-url)

*Fig. 2.*—Histogram showing the actual distribution of *V. dahliae* on the roots of five species (---) and the theoretical values for the Poisson distribution (---). (a) Common thornapple; (b) wheat; (c) soybean; (d) cotton; (e) Noogoora burr.

Measurements on cotton, soybean, Noogoora burr, common thornapple, wheat, barley, and grain sorghum showed that the colonies average about 2·0 mm in length. The experiment did not provide sufficient data to allow a valid comparison of the length of the colonies on the plants examined, but certainly there was no
evidence that the colonies were larger on those species which are colonized most frequently.

(ii) Distribution of *V. dahliae* on Plant Roots

Roots of soybean, cotton, Noogoora burr, and common thornapple were cut into 6-cm segments and 250 from each species were cultured for *V. dahliae*. Wheat roots, because they yield relatively few colonies per unit length, were cut into segments 10 cm in length and 200 were cultured for *V. dahliae*. The distribution of the fungus on the roots of these species was recorded and compared with the Poisson distribution using the $\chi^2$ goodness of fit test. For this test the observed and expected values were first grouped, as recommended by Kendall and Stuart (1961), into classes of approximately equal probability.

The observed distribution of *V. dahliae* on wheat, cotton, and soybean fitted the expected values within the limits set by the $\chi^2$ test, but the colonies were not at random on the roots of Noogoora burr and common thornapple, apparently because of crowding of the colonies on these two species. The experiments were repeated with a lower level of inoculum than in the *Verticillium*-infested soil. *Verticillium*-free soil was mixed 1 : 1 with infested soil and in the experiments with Noogoora burr and common thornapple the observed distributions fitted the theoretical values. The distribution of the fungus on all five species is shown in Figure 2 with the expected values for the Poisson distribution.

(c) Growth of *V. dahliae* on Plant Roots

An experiment was designed to measure the relative amount of growth of the fungus on the roots of five species which varied in susceptibility to systemic infection and proneness to colonization by *V. dahliae*.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Host reaction*</th>
<th>Index of colony size†</th>
<th>Plant</th>
<th>Host reaction*</th>
<th>Index of colony size†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat cv. Festiguay</td>
<td>I</td>
<td>20.75</td>
<td>Soybean cv. Lee</td>
<td>I</td>
<td>17.34</td>
</tr>
<tr>
<td>Cotton cv. Deltapine</td>
<td>S</td>
<td>18.05</td>
<td>Common thornapple†</td>
<td>S</td>
<td>19.24</td>
</tr>
<tr>
<td>Smoothleaf</td>
<td></td>
<td></td>
<td>Noogoora burr‡</td>
<td>S</td>
<td>34.87</td>
</tr>
</tbody>
</table>

* S, susceptible; I, immune.
† Expressed as number of colonies per 100 cm of fragmented root divided by number of colonies per 100 cm root (50 2-cm segments). Results are the mean of two experiments: standard error 3.33; least significant difference ($P = 0.05$) 9.43.
‡ To obtain good separation of the colonies on common thornapple and Noogoora burr these plants were grown in infested soil mixed 1 : 1 with *Verticillium*-free soil.

Five pots of wheat, cotton, soybean, Noogoora burr, and common thornapple were harvested and 100 segments of root were cut from the plants in each pot. Each sample was then divided into two subsamples of 50 segments and one lot was plated
on agar to obtain the number of colonies per unit length of root; the second lot was fragmented and the number of units of *V. dahliae* in the suspension was recorded. The ratio of the number of colonies per unit length of fragmented root to the number of colonies per unit length of intact root provided an index of the size of the colonies. The data of the first experiment indicated that the colonies on the roots of Noogoora burr might be larger than on the other species and the experiment was repeated. In the second experiment the size of the samples was doubled and eight observations were made on each treatment.

Analysis of the data from the two experiments, using the method of least squares, indicated that the colonies were significantly larger (*P*<0.05) on the roots of Noogoora burr than on the other four species (Table 2), but there was no evidence that colony size was correlated with susceptibility to systemic infection or proneness to colonization by *V. dahliae*. It appears, therefore, that factors affecting the growth of the fungus at the root surface are independent of factors affecting germination of the microsclerotia.

(d) Effect of Surface Disinfectants on the Number of Colonies of *V. dahliae* Surviving on Plant Roots

In previous experiments (unpublished) colonies of *V. dahliae* on plant roots were readily removed by low concentrations of surface disinfectants, which suggested that the fungus was restricted to the rhizoplane or to superficial infection sites in the root cortex. However, for a plant to become systemically infected by *V. dahliae*, the fungus must gain access to the vascular tissues and an experiment was designed to observe whether the colonies on susceptible species were less vulnerable to surface disinfectants than colonies on immune species. This was done by measuring the effect of increasing concentrations of mercury on the number of colonies of *V. dahliae* surviving on plant roots, expressing this relationship in linear form, and comparing the slope of these lines. The latter is used as an expression of the relative susceptibility of the colonies to the fungicide. Viewed in another way, the results would provide a measure of the relative susceptibility of the roots to penetration by *V. dahliae*.

One of the requirements of this experiment was that the initial level of colonization, i.e. the number of colonies per unit length of root, be equal for the species being studied. Cotton and soybean could be predicted to yield about 25 colonies per 100 cm of root and it was possible to achieve a similar level of colonization on the roots of Noogoora burr and common thornapple by diluting the infested soil with *Verticillium*-free soil. For Noogoora burr the mixture was three parts infested soil to two parts of *Verticillium*-free soil and for common thornapple the ratio was changed to two parts infested soil to three parts *Verticillium*-free soil. The level of inoculum was not adjusted to increase the number of colonies per unit length on wheat roots.

Six pots each of wheat, cotton, soybean, Noogoora burr, and common thornapple were harvested and the plants from three pots were bulked to provide duplicate samples of the five species. Seven hundred segments of root were cut from each sample and then divided into lots of 100 segments. One lot was plated in the usual way, the others were exposed for 1 min to aqueous solutions of mercuric chloride containing 2, 4, 8, 16, 32, and 64 p.p.m. mercury, rinsed in sterile water, and cultured to obtain the number of colonies of *V. dahliae* surviving on the roots.
The results of this experiment (Fig. 3), indicate high sensitivity of the colonies to low concentrations of mercury. The data expressing the relationship between mercury concentration and the number of colonies of *V. dahliae* surviving on plant roots appeared to fit a (negative) exponential of the form

\[ Y = Ae^{BX}, \]  

(1)

where \( Y \) is the number of colonies per 100 cm of root and \( X \) is the concentration of mercury (p.p.m.). This may be expressed in the form

\[ \ln Y = \ln A + BX, \]  

(2)

or

\[ Y' = A' + BX, \]  

(3)

where \( Y' = \ln Y \), \( A' = \ln A \), and \( A' \) and \( B \) were estimated by the method of least squares in a simple linear regression. Thus \( B \) represents the slope of the straight line fitted when \( Y' \) is plotted against \( X \) (in each case the regression was highly significant, \( P < 0.05 \)). By differentiating (1) with respect to \( X \), the gradient or slope of the curve

\[ \frac{dY}{dX} = ABe^{BX} \]  

(4)

is dependent on \( A \) (the initial level of colonization) as well as \( B \) and \( X \), and comparison of the slope of these lines is only valid so long as the initial level of colonization is the same.

Fig. 3.—Effect of mercury concentration on the number of colonies of *V. dahliae* surviving on the roots of five species.

- Wheat \( (y = 7.78e^{-0.007x}) \).
- Soybean \( (y = 26.41e^{-0.02x}) \).
- Cotton \( (y = 22.64e^{-0.03x}) \).
- Noogoora burr \( (y = 23.11e^{-0.03x}) \).
- Common thornapple \( (y = 24.66e^{-0.03x}) \).

The equations for the fitted curves were derived by retransforming the regression equations in Table 3.

Table 3 records the regressions expressing the effect of mercury concentration on the number of colonies of *V. dahliae* surviving per unit length of root on the five species and summarizes the results of the comparisons made between them. The slope of the regression for soybean was significantly different \( (P < 0.05) \) from cotton,
Noogoora burr, and common thornapple, but there were no significant differences between cotton, Noogoora burr, and common thornapple. Comparison of wheat with these four species was not valid since the initial level of colonization was significantly less than the other species. On the basis of these results there is no reason to reject the hypothesis that the colonies on soybean, an immune species, were more vulnerable to the mercury than were the colonies on three susceptible species, viz. cotton, Noogoora burr, and common thornapple. In this case the roots of the immune species were apparently more resistant to penetration by the fungus than the roots of three susceptible species, although on occasion V. dahliae has been isolated from immune species exposed to solutions containing up to 100 p.p.m. mercury.

### Table 3

**COMPARISON OF THE LINEAR REGRESSIONS EXPRESSING THE EFFECT OF MERCURY CONCENTRATION OF THE NUMBER OF COLONIES OF V. DAHLIAE SURVIVING PER 100 CM OF ROOT**

<table>
<thead>
<tr>
<th>Species</th>
<th>Regression equation</th>
<th>$R^2\dagger$</th>
<th>Test of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotton</td>
<td>$Y' = 3 \cdot 11995 - 0 \cdot 025X$</td>
<td>0.643</td>
<td>*</td>
</tr>
<tr>
<td>Soybean</td>
<td>$Y' = 3 \cdot 27387 - 0 \cdot 062X$</td>
<td>0.924</td>
<td>*</td>
</tr>
<tr>
<td>Wheat</td>
<td>$Y' = 2 \cdot 05097 - 0 \cdot 067X$</td>
<td>0.813</td>
<td>*</td>
</tr>
<tr>
<td>Noogoora burr</td>
<td>$Y' = 3 \cdot 14038 - 0 \cdot 030X$</td>
<td>0.822</td>
<td>*</td>
</tr>
<tr>
<td>Common thornapple</td>
<td>$Y' = 3 \cdot 20505 - 0 \cdot 032X$</td>
<td>0.860</td>
<td>*</td>
</tr>
</tbody>
</table>

### Species comparison

<table>
<thead>
<tr>
<th>Species comparison</th>
<th>Significance of difference in $B$</th>
<th>Significance of difference in $A'$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean, cotton</td>
<td>*</td>
<td>n.s.</td>
</tr>
<tr>
<td>Soybean, thornapple</td>
<td>*</td>
<td>n.s.</td>
</tr>
<tr>
<td>Soybean, Noogoora burr</td>
<td>*</td>
<td>n.s.</td>
</tr>
<tr>
<td>Cotton, thornapple</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Cotton, Noogoora burr</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Thornapple, Noogoora burr</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

* Significant ($P < 0.05$); n.s., not significant.

$\dagger 100R^2 = \text{percentage of total variation attributable to the fitted regression.}$

**IV. Discussion**

The results confirm an earlier report by Lacy and Horner (1966) that V. dahliae colonizes some species more frequently than others, although there is no evidence to support their thesis that, as an axiom, susceptible species are colonized more frequently than immune species. This discrepancy can be attributed to the small number of species examined by Lacy and Horner and emphasizes the need, in studies of this nature, to have a sufficiently large number of treatments to avoid misleading results. Again, there was no evidence that the susceptibility of eight cultivars of cotton to systemic infection was correlated with their proneness to colonization by V. dahliae. However, some soybean cultivars yielded more colonies per unit length than others, indicating the possibility that, within a species, the susceptibility of some lines or cultivars to systemic infection may be influenced by their proneness to colonization as well as their inherent resistance to wilt.
Once established on a root *V. dahliae* grows no better on species which yield a high number of colonies per unit length of root than on other species, indicating that factors influencing colonization are independent of factors influencing growth at the root surface. Thus the nutritional effect of the plant root on germination of the microsclerotium is believed to be the main reason why some species are colonized more frequently than others. Schreiber and Green (1963) reported that the exudate from tomato root was a more effective source of nutrient for stimulating the germination of conidia and microsclerotia inhibited by soil fungistasis than the exudate from wheat. In experiments reported here tomato roots were consistently colonized more frequently than wheat.

In soil *V. dahliae* survives as microsclerotia, either singly or in small groups embedded in fragments of plant tissue (Evans et al. 1966). Lacy and Horner (1966), Menzies and Griebel (1967), Green and Papavizas (1968), Emmatty and Green (1969), and Farley et al. (1971) report that when these propagules germinate there is a two- to threefold increase in inoculum density which is thought by some (e.g. Emmatty and Green) to increase the potential of the fungus to cause infection. Our findings that *V. dahliae* is distributed at random on roots does not seem to support this viewpoint. If microsclerotia distributed at random in soil germinated to form secondary inocula the resulting colonies might be expected to be clustered and not distributed at random. The experiments reported here show the colonies to be small discrete units, about 2·0 mm in length, each of which appears to arise from a different microsclerotium. This is not to suggest that a microsclerotium germinates only once. Menzies and Griebel (1967) and Farley et al. (1971) have shown that microsclerotia may germinate many times, and having done so in the presence of a root, a propagule could return to a quiescent state until it again came under the stimulus of another root. In this way germination could be repeated many times until the energy reserves of the microsclerotium were exhausted.

Most of the colonies on plant roots appear to be restricted to the rhizoplane or to superficial infection sites in the cortex where they are readily removed by exposure to low concentrations of mercury. Lacy and Horner (1966) and Evans (1971) observed that the colonies began to die on the roots within a few weeks after colonization and, except in plants that became systemically infected, the fungus was isolated only infrequently from dead roots. These observations suggest that colonies which fail to gain access to the vascular tissues of susceptible plants do not maintain the fungus, although on occasion some colonies on immune species survive even after the roots have been exposed to solutions containing 100 p.p.m. mercury. Most likely it is these colonies which Evans (1971) reported could survive in dead roots, but whether they serve to perpetuate the fungus for extended periods in the absence of susceptible plants is open to question. If, through the action of stimulating microsclerotia to germinate, immune species reduce the energy of the propagules and their resultant longevity, then the survival of a small number of colonies would not contribute to the persistence of the fungus. Immune plants might then be used as decoy crops to reduce the longevity of microsclerotia at a greater rate than would occur under bare fallow. The most effective decoy crops might be selected on the ability of the roots to stimulate germination of the *V. dahliae* propagule.
V. References


