A PRELIMINARY LABORATORY ASSESSMENT OF FUNGICIDES FOR THE CONTROL OF SCLEROTINIA SCLEROTIORUM (LIB.) D BY.

by Shaun R. Pennycook* and James B. Corbin*

SUMMARY

A poisoned food technique was used to test the efficacy of the organic fungicides benomyl, dicloran, quintozone, and difolatan against single hyphal-tip lines of a "Major" and a "Minor" strain of Sclerotinia sclerotiorum, isolated from diseased field tomatoes at Mangere. Dosage-response curves, derived from hyphal growth inhibition data over an initial 71-72 hr. period, are presented for the fungicides.

Over the initial 71-72 hr. period, benomyl and quintozone gave the lowest ED_{50} (0.45 ppm), and benomyl the lowest ED_{95} (1.3 ppm), against the "Major" strain; quintozone gave the lowest ED_{50} (0.36 ppm), and dicloran the lowest ED_{95} (11 ppm), against the "Minor" strain. For both strains, over 21 days, quintozone was most effective in inhibiting the formation of sclerotia and benomyl was most effective in inhibiting hyphal growth.

INTRODUCTION

Sclerotinia sclerotiorum (Lib.) D By is a widespread and omnivorous Discomycete pathogen, attacking forage legumes such as clovers and lucerne, a small number of fruit crops, and a very wide array of vegetable crops both in the field and in storage (Walker, 1952; Dickson, 1956; Walker, 1969). In New Zealand, it has been reported on more than 80 hosts (Dingley, 1969), and causes losses in crops such as tomato, potato, cabbage, lettuce and bean (Brien & Dingley, 1956).

The taxonomic status of the species is controversial. In the present work, the view of Purdy (1955) has been followed, that the proposed species S. sclerotiorum (Lib.) D By sensu stricto, S. trifoliorum Ericks., S. minor Jagger, S. intermedia Ramsey, S. sativa Drayt. & Groves, and S. trifoliorum Ericks. var. labae Keay, should all be included in a variable species S. sclerotiorum with sclerotial size designated by horticultural variety, "Major", "Intermedia" or "Minor".

No fully effective method of field control of the sclerotinia disease has yet been found. Varying degrees of success have been reported (Darby, 1961; Partyka & Mai, 1962; Grover, 1964; Beckman & Parsons, 1965; Natti 1967; Niedbalski & Rickard, 1969; Besemer et al, 1969) with field and laboratory applications of the four organic fungicides tested in the present work.

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The poisoned food technique of fungicide testing, i.e. comparison of the radial growth rates of the fungus on substrates impregnated with different concentrations of the fungicide, was largely developed for work with wood-rotting fungi (Horsfall, 1956), but it is equally useful with organisms which either do not sporulate at all, or cannot readily be induced to do so in the laboratory (Torgeson, 1967).

**MATERIALS AND METHODS**

**Organisms and Isolation Technique.**

Two strains of the fungus were isolated from a Mangere tomato crop in March, 1969. Numerous black sclerotia were obtained from the pith cavities of stems showing advanced symptoms of the sclerotinia disease. Of 25 6 to 8 inch segments of stem collected, 23 contained sclerotia all of which measured 3 to 8 mm by 3 to 5 mm, designated as *S. sclerotiorum* "Major", while the remaining two contained only spheroidal sclerotia measuring from 0.5 to 1 mm in diameter, designated as *S. sclerotiorum* "Minor". The sclerotia were surface sterilised for 5 minutes in a 1% sodium hypochlorite solution, and grown out at 17° C on 1.5% potato-dextrose agar (PDA), containing 20 g of glucose, and the extract from 200 g of potatoes, per litre. Portions of the resultant mycelia were transferred to 1.5% tap-water agar (WA), and incubated at 17°. Single hyphal-tips were excised from WA plates under a stereo dissecting microscope, and transferred to PDA; one such isolate from each strain was used to give stock cultures and a source of mycelial inoculum for experimentation.

**Fungicides**

Four commercial organic fungicides were tested:

i. **Benlate.** (Du Pont).
Active ingredient, 50% w/w benomyl. (*Methyl-1-(butylcarbamoyl)-2-benzimidazolcarbamate*).

ii. **Allisan.** (Boots).
Active ingredient, 50% w/w dicloran. (*2, 6-Dichloro-4-nitroaniline*).

iii. **PCNB.** (Frank Winstone).
Active ingredient, 75% w/w quintozene. (*Pentachloronitrobenzene*).

iv. **Ortho Difolatan 80 W.P.** (Chevron Chemical).
Active ingredient, 80% w/w difolatan. (*Cis-N [(1,1,2,2-tetrachloroethyl) thio] - 4-cyclohexene-1,2-dicarboximide*).
Experimental Method

PDA was prepared at one and one-quarter x strength and dispensed with a sterile syringe in 40 ml portions into sterile 100 ml Erlenmeyer flasks. With the agar kept molten at ca. 45-50° C, 10 ml of an appropriate fungicide suspension was pipetted into each flask to give media with final concentrations of 0.1, 1, 10 and 100 ppm of active ingredients for each fungicide. A control medium was prepared by adding 10 ml of sterile distilled water to another flask of agar. As each mixture was made, the flask was shaken thoroughly and the medium poured into five Petri dishes (85 mm diameter), ca. 10 ml per dish. Inoculum plugs were cut with a No. 1 cork-borer (ca. 4 mm diameter) from a single PDA plate culture of the fungal strain being used for the particular experiment. A plug was placed, mycelial surface downwards, in the centre of each agar plate. Incubation was at 17° C. Growth rates were ascertained by measuring each culture along two pre-marked diameters at right angles, at intervals, until the faster growing treatments covered the entire plate. For S. sclerotiorum "Major" these measurements were at 18, 25, 41, 52, 66 and 71 hr. incubation, and for S. sclerotiorum "Minor" at 19, 38, 50, 61 and 72 hr. The numbers of sclerotia per plate were counted after 21 days.

Statistical Methods

The method of Vincent (1947) was used in determining percent inhibition of radial growth, as it is unaffected by variations between treatments in the length of the lag phase. The radial growth increment between consecutive measurements was found for each plate, and a mean increment for each treatment calculated. The cumulative mean increment was graphed against time elapsed, and the mean growth rate in mm/24 hr. was estimated from the linear phase of the graph. Percent inhibition is given by:

\[ I = \frac{100 (C - T)}{C} \]

where C is the mean growth rate of the control, and T that of the treatment.

A more or less linear dosage-response curve for each fungicide was drawn by plotting percent inhibition against log concentration on probability paper; this is equivalent to the plot of the probit transformation of inhibition against log concentration (Horsfall, 1956). The effective doses for 50% inhibition (ED₅₀), for 84% inhibition (ED₈₄), and for 95% inhibition (ED₉₅), were obtained by interpolation from the dosage-response curves, and the slope in units of probits of inhibition per log concentration unit was calculated as:

\[ \text{Slope} = \frac{1}{\log_{10} \frac{\text{ED}_{84}}{\text{ED}_{50}}} \] (Horsfall, 1956).
TABLE 1: Mean growth rates and percent inhibitions after 71 hr. and sclerotial production and mean culture diameters after 21 days, of *S. sclerotiorum* "Major" cultures on poisoned food.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Growth Rate (mm/24hr.)</th>
<th>Percent Inhibition</th>
<th>Number of Sclerotia per Plate</th>
<th>Culture Diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>18.5</td>
<td>5.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benlate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 ppm</td>
<td>18.4</td>
<td>0</td>
<td>7.2</td>
<td></td>
</tr>
<tr>
<td>1 ppm</td>
<td>2.2</td>
<td>88.2</td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td>10 ppm</td>
<td>0.7</td>
<td>96.2</td>
<td>0</td>
<td>8.6</td>
</tr>
<tr>
<td>100 ppm</td>
<td>0.5</td>
<td>97.1</td>
<td>0</td>
<td>7.1</td>
</tr>
<tr>
<td>Allisan</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 ppm</td>
<td>19.4</td>
<td>0</td>
<td>6.6</td>
<td></td>
</tr>
<tr>
<td>1 ppm</td>
<td>10.6</td>
<td>42.7</td>
<td>7.4</td>
<td></td>
</tr>
<tr>
<td>10 ppm</td>
<td>0</td>
<td>100</td>
<td>0.6</td>
<td>31.6</td>
</tr>
<tr>
<td>100 ppm</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>10.3</td>
</tr>
<tr>
<td>PCNB</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 ppm</td>
<td>18.5</td>
<td>0</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td>1 ppm</td>
<td>2.0</td>
<td>89.1</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>10 ppm</td>
<td>1.5</td>
<td>91.7</td>
<td>0</td>
<td>37.6</td>
</tr>
<tr>
<td>100 ppm</td>
<td>1.2</td>
<td>93.3</td>
<td>0</td>
<td>31.1</td>
</tr>
<tr>
<td>Difolatan</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 ppm</td>
<td>18.5</td>
<td>0</td>
<td>4.6</td>
<td></td>
</tr>
<tr>
<td>1 ppm</td>
<td>11.3</td>
<td>38.7</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td>10 ppm</td>
<td>3.0</td>
<td>83.7</td>
<td>0</td>
<td>21.1</td>
</tr>
<tr>
<td>100 ppm</td>
<td>0.9</td>
<td>95.2</td>
<td>0.6</td>
<td>8.3</td>
</tr>
</tbody>
</table>

RESULTS

Tables 1 and 2 show mean growth rates and percent inhibitions for the various treatments as determined over the initial 71-72 hr. of incubation; and mean numbers of sclerotia per plate and mean culture diameters after 21 days' incubation.

Figures 1 and 2 show the dosage-response curves for the initial 71-72 hr. of incubation, drawn from the percent inhibition data presented in Tables 1 and 2.
TABLE 2: Mean growth rates and percent inhibitions after 72 hr. and sclerotial production and mean culture diameters after 21 days, of *S. sclerotiorum* ‘Minor’ cultures on poisoned food.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Growth Rate (mm/24hr.)</th>
<th>Percent Inhibition</th>
<th>Number of Sclerotia per Plate</th>
<th>Culture Diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13.9</td>
<td>------</td>
<td>329.3</td>
<td>------</td>
</tr>
<tr>
<td>Benlate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 ppm</td>
<td>13.5</td>
<td>0</td>
<td>343.4</td>
<td>----</td>
</tr>
<tr>
<td>1 ppm</td>
<td>11.6</td>
<td>17.3</td>
<td>387.0</td>
<td>----</td>
</tr>
<tr>
<td>10 ppm</td>
<td>1.4</td>
<td>89.8</td>
<td>0</td>
<td>26.5</td>
</tr>
<tr>
<td>100 ppm</td>
<td>0.6</td>
<td>96.0</td>
<td>0</td>
<td>6.9</td>
</tr>
<tr>
<td>Allisan</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 ppm</td>
<td>13.5</td>
<td>0</td>
<td>366.6</td>
<td>----</td>
</tr>
<tr>
<td>1 ppm</td>
<td>10.2</td>
<td>27.0</td>
<td>482.8</td>
<td>----</td>
</tr>
<tr>
<td>10 ppm</td>
<td>0.9</td>
<td>93.5</td>
<td>81.0</td>
<td>52.5</td>
</tr>
<tr>
<td>100 ppm</td>
<td>0</td>
<td>100</td>
<td>19.0</td>
<td>20.1</td>
</tr>
<tr>
<td>PCNB</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 ppm</td>
<td>13.0</td>
<td>6.8</td>
<td>376.8</td>
<td>----</td>
</tr>
<tr>
<td>1 ppm</td>
<td>1.6</td>
<td>88.5</td>
<td>193.6</td>
<td>71.6</td>
</tr>
<tr>
<td>10 ppm</td>
<td>0.8</td>
<td>94.5</td>
<td>0</td>
<td>27.6</td>
</tr>
<tr>
<td>100 ppm</td>
<td>0.6</td>
<td>95.5</td>
<td>0</td>
<td>23.6</td>
</tr>
<tr>
<td>Difolatan</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 ppm</td>
<td>13.3</td>
<td>4.6</td>
<td>326.0</td>
<td>----</td>
</tr>
<tr>
<td>1 ppm</td>
<td>8.7</td>
<td>37.7</td>
<td>254.2</td>
<td>----</td>
</tr>
<tr>
<td>10 ppm</td>
<td>3.1</td>
<td>77.6</td>
<td>0</td>
<td>39.6</td>
</tr>
<tr>
<td>100 ppm</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>9.6</td>
</tr>
</tbody>
</table>

Tables 3 and 4 show the dosage-response statistics (ED\textsubscript{50}, ED\textsubscript{84}, ED\textsubscript{95}, and slope) derived from Figures 1 and 2.

Over the initial 71-72 hr. Allisan, PCNB, and Difolatan gave a similar degree of hyphal inhibition against *S. sclerotiorum* ‘Minor’ as against *S. sclerotiorum* ‘Major’. Benlate was much less effective against *S. sclerotiorum* ‘Minor’ (ED\textsubscript{50} = 2.6 ppm) than against *S. sclerotiorum* ‘Major’ (ED\textsubscript{50} = 0.45 ppm). (Figures 1 and 2, Tables 3 and 4).

Of the four fungicides, only Benlate and PCNB completely inhibited sclerotial production over 21 days at 10 and 100 ppm of fungicide. Allisan at 1 ppm stimulated sclerotial production. (Tables 1 and 2).
FIG. 1: Dosage-response curves from *S. sclerotiorum* "Major" poisoned food tests.

TABLE 3: Dosage-response statistics from *S. sclerotiorum* "Major" poisoned food tests.

<table>
<thead>
<tr>
<th>Fungicide</th>
<th>$ED_{50}$ (ppm)</th>
<th>$ED_{84}$ (ppm)</th>
<th>$ED_{95}$ (ppm)</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benlate</td>
<td>0.45 ppm</td>
<td>0.87 ppm</td>
<td>1.3 ppm</td>
<td>3.4</td>
</tr>
<tr>
<td>Allisan</td>
<td>1.2 ppm</td>
<td>3.1 ppm</td>
<td>5.8 ppm</td>
<td>2.4</td>
</tr>
<tr>
<td>PCNB</td>
<td>0.45 ppm</td>
<td>0.87 ppm</td>
<td>3000 ppm*</td>
<td>3.4</td>
</tr>
<tr>
<td>Difolatan</td>
<td>1.7 ppm</td>
<td>10.2 ppm</td>
<td>94 ppm</td>
<td>1.3</td>
</tr>
</tbody>
</table>

* by extrapolation.
FIG. 2: Dosage-response curves from *S. sclerotiorum* "Minor" poisoned food tests.

TABLE 4: Dosage-response statistics from *S. sclerotiorum* "Minor" poisoned food tests.

<table>
<thead>
<tr>
<th>Fungicide</th>
<th>ED&lt;sub&gt;50&lt;/sub&gt; (ppm)</th>
<th>ED&lt;sub&gt;84&lt;/sub&gt; (ppm)</th>
<th>ED&lt;sub&gt;95&lt;/sub&gt; (ppm)</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benlate</td>
<td>2.6 ppm</td>
<td>7.4 ppm</td>
<td>59 ppm</td>
<td>2.2</td>
</tr>
<tr>
<td>Allisan</td>
<td>1.9 ppm</td>
<td>5.6 ppm</td>
<td>11 ppm</td>
<td>2.1</td>
</tr>
<tr>
<td>PCNB</td>
<td>0.36 ppm</td>
<td>0.85 ppm</td>
<td>25 ppm</td>
<td>2.7</td>
</tr>
<tr>
<td>Difolatan</td>
<td>2.1 ppm</td>
<td>13.8 ppm</td>
<td>45 ppm</td>
<td>1.2</td>
</tr>
</tbody>
</table>
The complete inhibition of hyphal growth given initially by Allisan at 10 and 100 ppm (Tables 1 and 2) broke down after ca 14 days in most plates. In some cases the ensuing growth rate approached that of the controls. The mutagenic potential of this fungicide on *Gilbertella persicaria* has been noted by Ogawa et al (1963), but in the present instance, with the rapid growth occurring round the entire circumference of the culture rather than in a segment, physiological adaptation may be a more likely explanation than mutation.

Difolatan at 10 and 100 ppm stimulated the production from the inoculum plug of a mass of aerial hyphae, which in many instances spread the full width of the Petri dish.

**DISCUSSION**

From the data presented, four criteria can be used to rank the performances of the fungicides in order of decreasing effectiveness:

i. **ED$_{50}$** of inhibition of hyphal growth during the initial 71-72 hr. Against *S.sclerotiorum* "Major"; Benlate > PCNB > Allisan > Difolatan. Against *S.sclerotiorum* "Minor"; PCNB > Allisan > Difolatan > Benlate.

ii. **ED$_{95}$** of inhibition of hyphal growth during the initial 71-72 hr. Against *S.sclerotiorum* "Major"; Benlate > Allisan > Difolatan > PCNB. Against *S.sclerotiorum* "Minor"; Allisan > PCNB > Difolatan > Benlate.

iii. Inhibition of sclerotal production over 21 days. PCNB > Benlate > Difolatan > Allisan.

iv. Inhibition of hyphal growth over 21 days. Benlate > Difolatan > Allisan > PCNB.

The fourth criterion is probably the most meaningful of the four as an indication of the likely efficacy of the fungicides in controlling mycelial growth. The third criterion may also be of some significance in crop protection, since the sclerotia are not only a highly resistant resting stage carrying the pathogen over between crops, but also the source of apothecia and hence of the ascospores which are a major inoculum source.

The dosage-response curves obtained (Figures 1 and 2) correspond to two of the four forms described by McCallan et al (1941), viz. a simple straight line, and a compound slope, convex upwards. The **ED$_{50}$** is the most precisely determined point on a log-probability dosage-response curve, and is generally favoured as the level of comparison for compounds giving simple straight line curves of similar slope. However, an **ED$_{50}$** comparison is inadequate when comparing compounds giving curves of
differing slope, and particularly those giving 'convex' curves, i.e. those with considerably reduced slope in the upper dosages. A more meaningful comparison in such cases can be made at a higher level such as ED<sub>95</sub> (McCallan et al, 1941). In the present work, the effect of changing from an ED<sub>50</sub> to an ED<sub>95</sub> comparison is to demote PCNB by one or two ranks, giving a ranking of the fungicides closer to that obtained on the basis of hyphal inhibition over 21 days. Since a comparison at some other arbitrary high ED level, e.g. ED<sub>90</sub> or ED<sub>99</sub>, gives yet another different ranking, it is probable that this technique is insufficiently powerful to separate the performances of these fungicides under the experimental conditions over a period of 71-72 hr.

A difference of slope of the dosage-response curves of different compounds was considered to indicate a difference in mode of action (Dimond et al, 1941; McCallan et al, 1941; Horsfall, 1956). More recent work (McCallan et al, 1959) has cast doubt on this hypothesis, so that no significance can be attached to the smaller slope of the Difolatan curves compared with those of the other three fungicides (Figures 1 and 2).

From the data presented, the new fungicide, Benlate, appears to warrant trial against <i>S. sclerotiorum</i> in the field. If PCNB is taken as the standard, Benlate is comparable for short term mycelial control, superior for long term mycelial control, and only slightly inferior for preventing sclerotial formation. If Allisan is taken as the standard, Benlate is superior by all criteria, except the short term control of mycelium of the "Minor" strain.

ACKNOWLEDGEMENT

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REFERENCES


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