Variation Among Isolates of *Macrophomina phaseoli* 
(*Rhizoctonia bataticola*) from Different Regions

By

O. D. DHINGRA and J. B. SINCLAIR
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Introduction

Variation among phytopathogens is common and must be elucidated if the behaviour on their respective hosts is to be understood. Variation among *Rhizoctonia* spp. other than *R. bataticola* (Taub.) Butler has been reviewed (FLENTJE et al. 1970). Isolates of *Macrophomina phaseoli* (Maubl.) Ashby (*R. bataticola*) pathogenic to several hosts have been reported to vary in their ability to produce enzymes (KULKARNI et al. 1971). *M. phaseoli* from the same host, whether urid bean (*Phaseolus mungo* [L.]), (KHARE et al. 1970) or soybean (*Glycine max* [L.] Merr.) (DHINGRA and SINCLAIR 1972) were shown to differ depending on the plant part from which it was isolated. This report shows soybean isolates of *M. phaseoli* from various regions in the U.S. vary within and between the collection areas.

Materials and Methods

Nine isolates of *M. phaseoli* from soybean were collected from the following states and labeled, respectively: Arkansas, A; Illinois 1-1, 1-2, 1-3; and Missouri, M-1, M-2, M-3, M-4, M-5. Single hyphal tip stock cultures were maintained and stored on Difco potato-dextrose agar (PDA) at 4 °C.

The in vitro variability for growth rate and colony characteristics of the isolates was studied on PDA at five temperatures (15, 20, 25, 30, and 35 °C) and on three media at 25 °C. The media used were: PDA, Difco nutrient agar (NA) and soybean-seed agar (SSA) (150 g seed/liter of water, boiled for 10 min, strained through cheese cloth, then adding 15 g Difco agar/liter). Agar discs (4 mm) were cut from the periphery of 2-day-old colonies with a sterile corkborer and transferred to the center of culture plates containing 15 ml of agar. Colony characteristics and diameters (in mm) were recorded after 48 hrs. Sclerotia size of each isolate was measured after 4 days at 25 °C on PDA.
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Pathogenicity of each isolate was studied using 'Amsyo' seedlings grown in vermiculite (Terralite brand) in a growth chamber programmed for 30 °C, 50 % relative humidity, with 14-hr day length; or in the greenhouse at 23—27 °C. For each experiment, 15 seedlings each were wound-inoculated with one of the isolate. The hypocotyl of all seedlings were inoculated 1 cm below the cotyledonal node 4 days after emergence. Inoculations were made using a sterile needle containing a mixture of mycelium and sclerotia from a 7-day-old culture of the test fungus. Wound-punctured, but noninoculated seedlings served as controls. Seedlings were observed daily for symptom development for 15 days. The number of seedlings killed and shoot length of survivors were recorded. Using PDA, isolations were made from surviving plants to determine the extent the fungus moved within inoculated seedlings.

For each study, the data from three generations, each replicated three times were combined for statistical analysis.

Results and Discussion

There was variation between the different isolates studied, but not within three generations for any single isolate. Variation was such that only general relationships were noted. The maximum growth of all isolates on PDA was 35 °C (fig. 1). Total growth of all isolates decreased with decrease in incubation temperature. There was variation between isolates within each temperature, nevertheless the isolates could be placed into three general categories based on growth rate: rapid: A-1, I-1, I-2, M-4; moderate: I-3, M-1, M-2, M-4, M-5; and slow: M-3.

Colony characteristics varied between isolates and incubation temperatures. The color of most isolates was brown. I-2, M-1 and M-3 had completely appressed growth with even colony margins at all temperatures on PDA, with M-3 producing irregular colony margins, turning from dark green with age with no sclerotia. M-2 produced a semi-fluffy at 35 °C, but appressed at 25 and 30 °C. A-1 and M-4 were white and fluffy at all temperatures. I-1 and I-3 produced fluffy colonies at 35 °C with less fluffiness with each decrease in temperature.

![Fig. 1. Mean *) radial growth, in mm, of nine isolates of *Macrophomina phaseoli* (*Rhizoctonia bataticola*) at five temperatures](image)

*) Mean of three generations with three replications each. The 1 % D for isolates at 1 % level = 2.36.
Growth rate differed among the isolates on the three media at 25°C (table). In general, all isolates produced maximum growth on PDA. The growth rate of the isolate all media tended to fall into three categories: rapid: M-2, M-4; moderate: A-1, I-1, I-3, M-1; and slow: M-3, M-5. I-2 produced no consistent pattern.

Table
Mean(*) radial diameter, in mm, of nine isolates of Macrophomina phaseoli (Rhizoctonia bataticola) after 48 hours growth on three media: Difco potato-dextrose agar (PDA), Difco nutrient agar (NA), and soybean seed extract agar (SSA) at 25°C

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Media</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>PDA</td>
<td>NA</td>
<td>SSA</td>
<td>Mean(*)</td>
</tr>
<tr>
<td>A-1</td>
<td>52.50</td>
<td>36.53</td>
<td>45.26</td>
<td>44.76</td>
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<tr>
<td>I-1</td>
<td>54.06</td>
<td>32.10</td>
<td>41.53</td>
<td>45.56</td>
</tr>
<tr>
<td>I-2</td>
<td>49.76</td>
<td>32.50</td>
<td>50.06</td>
<td>38.10</td>
</tr>
<tr>
<td>I-3</td>
<td>61.56</td>
<td>38.43</td>
<td>41.96</td>
<td>47.18</td>
</tr>
<tr>
<td>M-1</td>
<td>52.73</td>
<td>32.20</td>
<td>41.06</td>
<td>41.99</td>
</tr>
<tr>
<td>M-2</td>
<td>60.63</td>
<td>39.60</td>
<td>49.96</td>
<td>50.06</td>
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<td>M-3</td>
<td>18.20</td>
<td>11.06</td>
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<td>11.77</td>
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<tr>
<td>M-4</td>
<td>61.53</td>
<td>61.06</td>
<td>75.43</td>
<td>66.02</td>
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<tr>
<td>M-5</td>
<td>23.86</td>
<td>21.73</td>
<td>41.53</td>
<td>29.04</td>
</tr>
<tr>
<td>Mean</td>
<td>47.60</td>
<td>31.69</td>
<td>44.54</td>
<td></td>
</tr>
</tbody>
</table>

*) Mean of three generations with three replications each.

LSD at 1 % level: for isolates = 1.56; for media = 0.90; and for interaction = 2.73.

No isolates produced sclerotia of the same size. The average size in micron (length × width) of 25 sclerotia for each isolate was: A, 121 × 82; I-1, 97 × 84; I-2, 291 × 193; I-3, 132 × 102; M-1, 88 × 76; M-2, 105 × 95; M-3, 50 × 44; M-4, 113—102; and M-5, 121 × 82. A-1, I-2 and I-3 produced irregular-shaped sclerotia, while all others were oblong-to elliptical-shaped sclerotia. Only isolate A-1 produced a light-colored outer layer of bulbils.

The pathogenicity of the isolates varied within the two temperatures. At 30°C, the virulent isolates (percent seedlings killed) were: A-1 (83), I-1 (73), I-2 (67), M-1 (83), and M-2 (80); moderately virulent isolates were: M-4 (53); and the weakly virulent were I-3 (0), M-3 (0), M-5 (0). The fungus was reisolated from all seedlings, except those inoculated with M-3 and the controls. Under greenhouse conditions, the pattern was similar, but the mortality lower. The more virulent isolates caused stunting of surviving seedlings. Seedlings inoculated with I-2 and M-4 developed rosetting of the epicotyl (fig. 2) and pycnidia developed on dead seedlings. I-2 produced pycnidia in the greenhouse and growth chamber, M-4 produced pycnidia only in the greenhouse, and M-3 only around the point of inoculation both in the greenhouse and growth chamber.

There appeared to be some relationship between in vitro growth rate at 30°C and virulence. These results confirm those of Watson (1944), Edmund (1964) and Vasudeva and Ashraf (1939) that isolates of R. bataticola from potato, grain sorghum, and cotton, respectively, were most virulent at high
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Fig. 2. Soybean seedlings noninoculated (left) or inoculated with either isolate 16 (Illinois-2) (center) or 15 (Missouri-4) (right) of *Macrophomina phaseoli* (*Rhizoctonia bataticola*) showing stunting and rosetting effect which is more severe with isolate M-4.

temperatures (30 °C or above). JAIN (1969) related growth rate of *R. bataticola* to virulence.

That different isolates of *M. phaseoli* from the same host plant may produce different symptoms was shown for the first time. The symptoms ranged from seedling blight through stunting and rosetting to death of soybean seedlings. Symptoms of seedling blight on stem-inoculated soybean were similar to those observed by W. A. MEYER (personal communication, 1971) in India on inoculated or naturally-infected soybean seedlings, and to symptoms produced on other infected legume seedlings (JAIN 1971, PHILIP et al. 1969). Rosetting and pycnidial formation was observed for the first time on stem-inoculated soybean seedlings. Pycnidial formation and pathogenicity were not correlated.

These differences in isolates of the test fungus may help to explain in part differences in experimental results among research workers.

Summary

Nine isolates of *Macrophomina phaseoli* (*Rhizoctonia bataticola*) from soybean were collected from three geographic areas in the U.S. Single hyphal tip cultures were studied for their variation in cultural characteristics and virulence in causing soybean seedling blight. All isolates varied in their growth rate and colony characteristics on PDA at 15, 20, 25, 30 and 35 °C. Maximum growth of seven isolates was at 35 °C and two at 30 °C. Growth rates and sclerotia varied on three media at 25 °C. The isolates varied from virulent, through moderately virulent, to weakly virulent. Pycnidia formation and rosetting on inoculated seedlings was recorded for the first time. There was a general correlation between in vitro growth on PDA and virulence of the
isolates. The studies were carried out for three generations using single, hyphal-tip isolates. There was no variation within any single isolate.

Zusammenfassung

Unterschiede zwischen verschiedenen Isolierungen von *Macrophomina phaseoli* (*Rhizoctonia bataticola*)


Literature


Authors' address: Department of Plant Pathology, University of Illinois, Urbana, Illinois 61801 (U.S.A.).

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