EVALUATION OF METHODS FOR INOCULATING BEANS WITH RUST UREDIOSPORES

J. V. Cordoba, J. R. Steadman, and D. T. Lindgren
University of Nebraska-Lincoln
Lincoln, NB 68503

Researchers studying bean rust have used various inoculation methods for differentiation of races of the fungus and screening lines for rust resistance. Our initial efforts to employ some of these methods were of limited success. Thus, five methods of inoculating Phaseolus vulgaris cv. Pinto 114 with rust (Uromyces phaseoli typica) were evaluated. The inoculum consisted of 1) a suspension of urediospores, 10 ml of tap water, and one drop of Tween-20; 2) a mixture of urediospores and 1 g of talc; 3) a suspension of urediospores and 10 ml of oil (Philips Soltrol 170); 4) a suspension of urediospores and 10 ml of distilled water; and 5) dry urediospores. Seedling inoculations were made approximately 10 days after planting when the primary leaves had reached about two-thirds of their normal size. About 0.1 g of freshly collected urediospores were used in each treatment. Tests were conducted in a randomized complete block design containing five replicates. Treatments 1, 2, 3, and 4 were applied with a Devilbiss No. 127 atomizer to the primary leaves of 10 bean seedlings per treatment. In addition, seedlings in treatment 2 were sprayed lightly with water previous to the inoculation, and the spore suspension in treatment 4 was agitated vigorously immediately before spraying.

Inoculation with a spatula, treatment 5, was made by transferring the urediospores from a small beaker to premoistened leaves with a spatula by gentle rubbing. After an incubation period of 18 hours in a moist chamber maintained at 18°C, the plants were transferred to a greenhouse bench.

The number of pustules on each leaf was counted 10 days after inoculation. Mean differences were compared using Duncan's Multiple Range Test. (Table 1)

The most severe infection of leaves resulted from the spatula method, treatment 5. Aggregation of spores was observed with this method, and as a result the size of the pustules varied because of multiple infections. Rust symptoms appeared earlier with this method than with others.

Moderate numbers of uniform pustules were achieved by spraying a mixture of talc and urediospores, treatment 2. No aggregation of spores was observed and the size of the mature pustules was uniform.

The inoculation technique using tap water and Tween-20, treatment 1, showed significantly lower number of pustules than when plants were inoculated with the spatula or talc methods. The distribution of spores, however, and the size of the pustules were uniform.

Inoculation of spores with distilled water as a suspending agent, treatment 4, produced fewer pustules than treatments 1, 2, or 3. As with the spatula method, water inoculation did not result in an even distribution of spores. Some aggregation of spores resulted in variation in the size of pustules.

Treatment 3, which employed Philips Soltrol 170 as a suspending agent,
caused considerable injury to the leaves and, in addition, produced a low number of pustules.

Treatment 2, using a mixture of talc and urediospores, was considered to be the most effective and was used in further studies to determine the reaction of edible dry bean cultivars to inoculation with rust spores.

Table 1. Mean number of rust pustules per bean leaf of plants inoculated by five methods.

<table>
<thead>
<tr>
<th>Treatment (NO.)</th>
<th>Mean No. of Pustules/Leaf</th>
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</thead>
<tbody>
<tr>
<td>Urediospores spread with spatula (5)</td>
<td>286 a</td>
</tr>
<tr>
<td>Urediospores + talc (2)</td>
<td>174 b</td>
</tr>
<tr>
<td>Tween-20 + urediospores + tap water (1)</td>
<td>127 c</td>
</tr>
<tr>
<td>Urediospores + distilled water (4)</td>
<td>95  d</td>
</tr>
<tr>
<td>Urediospores + oil¹ (3)</td>
<td>65  e</td>
</tr>
</tbody>
</table>

¹ Means followed by a common letter are not significantly different at the 0.05 level.
² Philips Soltrol 170

IDENTIFICATION OF PHYSIOLOGICAL RACES OF BEAN RUST IN WESTERN NEBRASKA AND NORTHEASTERN COLORADO

J. V. Cordoba, J. R. Steadman, and D. T. Lindgren
University of Nebraska-Lincoln
Lincoln, NB 68503

The presence of the bean rust fungus and physiologic races in Nebraska was reported in 1951; however, no race identification studies have been conducted since then. Five isolates of rust from western Nebraska and one from northeastern Colorado were derived from single pustules, increased, and inoculated to the seven Harter and Zaumeyer (1941) differential bean cultivars listed in Table 1. The grading system of Davison and Vaughan (1963) was used in all cases. In addition, Golden Gate Wax, used by Fisher (1952) also was inoculated. Based on the data in Table 1, it would appear that one physiologic race, race 25, was present in some of the Nebraska isolates (i.e. D and F). Isolates B, C, and E may be race 25 or a very similar new race. Isolate A could be race 20 or race 25. Difficulty in separating grades, especially 4 and 5, with a printed rust grading guide makes race identification difficult. Even measuring pustule size with an ocular micrometer did not improve grade separation between 4 and 5. In addition, only Golden Gate Wax was resistant to the race(s). The susceptibility of all the other differential cultivars made the identification of the bean rust race difficult. Future investigations of bean rust identification should include more differential cultivars which will have worldwide acceptability and some method of reducing the number.

All cultivars of pinto and great northern bean types grown in Nebraska are susceptible to rust races except a Colorado breeding line, 3385 which was