The Effect of Inoculum Levels on Field Evaluations of Potatoes for Verticillium Wilt Resistance

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Accepted for publication 3 September 1974.

ABSTRACT

Inoculum levels of Verticillium albo-atrum can be adjusted to develop different populations of the pathogen in the soil throughout the growing season. Severity of Verticillium wilt on potatoes is related to the inoculum density during tuberization, and to the environment. The wilt resistance of the 12 potato cultivars used in this test could be modified by alteration of the inoculum concentration. Even the highest level of wilt resistance available could be broken by high populations of the pathogen. Actual populations of the pathogen in the soil of the growing region should be compared to those developed in test plots to insure meaningful ratings of disease reaction.

Additional key words: population density, clones.

Phytopathology 65:225-228

For many years the U.S. Department of Agriculture (USDA) potato-breeding program has conducted field tests to evaluate the reaction of potato clones inoculated with Verticillium albo-atrum Reinke & Berth. (nonsclerotial form) on Aroostook Farm, Presque Isle, Maine. The tests have been conducted on land maintained on a 3-year rotation schedule of potatoes, grain, and sod, thus the population of Verticillium is relatively low as the test year begins. To inoculate plants, seed pieces are dipped into a Verticillium spore suspension of $8 \times 10^5$ spores/ml (2). In evaluation of these tests, the relationship has not been emphasized between
TABLE 1. *Verticillium* populations sampled from a plot planted with seed pieces inoculated with five levels of *Verticillium albo-atrum* in 1972 and 1973

<table>
<thead>
<tr>
<th>Spore concentration of seedpiece dip (× 10^5/ml)</th>
<th><em>Verticillium</em> populationa,b (propagules × 10^5/g soil)</th>
<th>1972</th>
<th>1973</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>39 v</td>
<td>22 v</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>62 w</td>
<td>47 w</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>91 x</td>
<td>54 w</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>120 y</td>
<td>96 x</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>118 y</td>
<td>100 x</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>170 z</td>
<td>117 y</td>
<td></td>
</tr>
</tbody>
</table>

a Average of four replications; samples taken from plant rhizosphere at 2-3 days before harvest.

b Duncan’s multiple-range test was used (P = 0.01). Treatments followed by the same letter do not differ significantly.

natural *Verticillium* levels in Aroostook soils and the inoculum level used in screening. Screening at high inoculum levels may detect lines with high levels of resistance, but many clones are discarded because the test is excessively severe.

TABLE 2. Comparison of *Verticillium albo-atrum* disease indices, inoculum levels, and soil populations taken from the rhizosphere of the cultivars Abnaki and Kennebec at various locations in Aroostook County, Maine

<table>
<thead>
<tr>
<th>Source of test</th>
<th>Year</th>
<th>Soil populationa (propagules × 10^5/g)</th>
<th>Disease index</th>
<th>Inoculum level (spores × 10^9/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Abnaki</td>
<td>Kennebec</td>
<td>Abnaki</td>
</tr>
<tr>
<td>USDA breeding plot</td>
<td>1972</td>
<td>115</td>
<td>110</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>1973</td>
<td>130</td>
<td>129</td>
<td>1.4</td>
</tr>
<tr>
<td>Verticillium inoculum-level plot (Table 3)</td>
<td>1972</td>
<td>90</td>
<td>91</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>1973</td>
<td>111</td>
<td>118</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>1973</td>
<td>109</td>
<td>110</td>
<td>1.0</td>
</tr>
<tr>
<td>Composite soil samples from Aroostook County</td>
<td>1972</td>
<td>42</td>
<td>85</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>1973</td>
<td>57</td>
<td>63</td>
<td>1.0</td>
</tr>
</tbody>
</table>

a Samples taken at time of harvest; average of four replications.

b Rated on a 1-5 scale; with 1 = no disease, and 5 = plant death.

TABLE 3. Disease reactions of 12 potato cultivars as affected by inoculum levels of *Verticillium albo-atrum* used to dip seed pieces in 1972 and 1973

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Inoculum levels (spores × 10^9) (1972)</th>
<th>Disease reactions following inoculation with:</th>
<th>Inoculum levels (spores × 10^9) (1973)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Abnaki</td>
<td>1.3 w</td>
<td>3.0 x</td>
<td>3.0 x</td>
</tr>
<tr>
<td>Houma</td>
<td>2.0 x</td>
<td>3.0 x</td>
<td>3.0 x</td>
</tr>
<tr>
<td>Katahdin</td>
<td>2.5 x</td>
<td>3.0 x</td>
<td>3.0 x</td>
</tr>
<tr>
<td>Kennebec</td>
<td>2.0 x</td>
<td>3.0 x</td>
<td>4.0 x</td>
</tr>
<tr>
<td>Monona</td>
<td>3.5 y</td>
<td>4.0 yz</td>
<td>4.5 yz</td>
</tr>
<tr>
<td>N. Rustic</td>
<td>3.5 y</td>
<td>3.5 xy</td>
<td>4.0 xy</td>
</tr>
<tr>
<td>Superior</td>
<td>4.5 z</td>
<td>4.5 z</td>
<td>5.0 z</td>
</tr>
<tr>
<td>Alamo</td>
<td>4.5 z</td>
<td>4.5 z</td>
<td>4.5 yz</td>
</tr>
<tr>
<td>Norland</td>
<td>...</td>
<td>NO TEST</td>
<td>...</td>
</tr>
<tr>
<td>Cobbler</td>
<td>...</td>
<td>NO TEST</td>
<td>...</td>
</tr>
<tr>
<td>Hug</td>
<td>4.5 z</td>
<td>4.0 yz</td>
<td>5.0 z</td>
</tr>
<tr>
<td>Sable</td>
<td>4.0 yz</td>
<td>4.5 z</td>
<td>4.5 yz</td>
</tr>
</tbody>
</table>

a Disease index on a 1-5 scale; 1 = no disease and 5 = plant death.

b Each value is an average of four replications, 10 hills per replication. Duncan’s multiple-range test was used (P = 0.01). Treatments followed by the same letter do not differ significantly.
10 × 10^4 spores/ml of solution. A control of distilled water served as the sixth treatment. The final concentrations were adjusted just before seed pieces were dipped.

For this test, 12 cultivars were chosen, with four cultivars classified as early, four as medium, and four as late. The cultivars were classified as resistant (R), intermediate (I), or susceptible (S), depending on their reactions in previous tests or disease reports. (The cultivars without disease ratings for Verticillium were not known or classified before this test.) The late cultivars were Abnaki (R), Houma (I), Katahdin (S), and Kennebec (S). The medium cultivars were Monona (I), Norgold Russet (S), Superior, and Alamo. The early cultivars were Norland (S), Irish Cobbler (S), Haig, and Sable.

The seed pieces were cut in the field, immediately dipped into their respective inoculum concentrations, planted in a 10-hill plot with 25-cm spacing, and covered immediately to prevent drying of the inoculum. The complete test consisted of the 12 cultivars, six treatments, and four replicates, totaling 288 ten-hill plots. The plots were fertilized with 15-15-15 at the rate of 21.6 kg N/hectare (ha) and treated as necessary with endosulfan (1.17 liters/ha), methyl demeton (1.75 liters/ha), and zine ion maneb (2.25 kg/ha). Plants were rated on a 0-5 scale, with 1 indicating no disease, and 5 indicating plant death. These ratings were made weekly after the first visible symptom of wilt was detected. Soil samples were taken in June and once each month through September from plots of each inoculum level (inoculum-level plot). Each sample consisted of a composite taken from each rhizosphere. The samples were assayed for Verticillium populations by use of a soil dilution-plate method.

Soil populations of V. albo-atrum were measured in the rhizosphere of Abnaki and Kennebec, grown in the USDA disease-resistance breeding plots in 1972 and 1973. The populations were composite samples, taken at several locations throughout the breeding plot where these cultivars were grown. The plants had been inoculated by a seedpiece dip, as previously described. For final disease index before harvest, the total indices of each cultivar were averaged. The final composite soil population of Verticillium was determined during this period, which corresponded to the date for evaluation of the inoculum-level plot.

Soil populations of V. albo-atrum were measured in the rhizosphere of Abnaki and Kennebec grown also on several farms in Aroostook County. The populations and disease indices (county average) were calculated as described above for the breeding plot.

RESULTS.—The inoculum levels added to the soil developed populations relative to their concentrations. The highest inoculum level resulted in the greatest number of Verticillium propagules/g of rhizosphere soil (Table 1). These populations corresponded to the levels found throughout Aroostook County soils in the past 3 years. In a general soil survey, the range of populations on 18 August, 1972, was from 51-120 × 10^4 propagules/g of rhizosphere soil and on 27 August, 1973, from 33-160 × 10^4 propagules/g of soil. The average populations in the rhizosphere of Abnaki and Kennebec are found in Table 2.

The late-maturing cultivars in the inoculum-level plot in 1973 showed differences in cultivar reactions; Abnaki was rated resistant; Houma, intermediate; Katahdin, susceptible; and Kennebec, most susceptible (Table 3). In the medium and early-maturing cultivars, there was no gross difference in their disease reactions. For example, in early-maturing cultivars, Norland rated 3.5 in 1973; Cobbler, 4.5; Haig, 3.5; and Sable, 4.0 when inoculated with 10 × 10^5 spores/ml. These cultivars were all classified as susceptible to Verticillium.

In 1972, there were no significant differences among the disease reactions between cultivars in each maturity class.

In the late-maturing cultivars, the delineation between resistance and susceptibility was not as apparent as it was in 1973.

Although the final disease indices were higher, Verticillium wilt developed more slowly in the inoculum level plot in 1972 than in 1973. The overall growing season was 2 weeks longer in 1973; thus the slow disease development was negated by a dry season before harvest. The disease ratings, even at the lowest inoculum level, were higher in 1972 than in 1973. Soil populations also were higher in 1973 at the final sampling (Table 1). Norland and Cobbler were removed from the test in 1972, because of excessive levels of leaf roll and spindle tuber virus.

Soil populations in the breeding plot and the inoculum level plot when 8 × 10^5 spores/ml of inoculum were added on the seed pieces, were higher than the average populations found throughout Aroostook County (Table 2). The disease indices in these plots also were generally higher than those found throughout the county. Abnaki, which is normally used as a resistant check in the annual USDA breeding plot, had similar ratings in both the breeding plot and the inoculum-level plot, and this was also evident with Kennebec, the susceptible check. However, for Abnaki, the breeding plot indices in 1972 and the higher inoculum level plot index in 1973 are considerably higher than the average indices for the county in the same years.

DISCUSSION.—Verticillium wilt resistance evaluations can be very complex and tedious if all facets of the disease syndrome are evaluated, including stem end browning, vascular discoloration, and foliar wilting. After several years of evaluation of wilt resistance by measurement of these factors, it appears that the extent of foliar symptoms can be used to accurately appraise resistance. This has been true in the USDA disease evaluations for the National Potato Breeding Program. If the foliar symptoms are compared with the symptoms of check cultivars throughout the plot, the susceptible clones can be eliminated, and resistant materials may be rescreened the next year. The primary problem has been the selection of a proper inoculum level for an accurate measure of resistance.

For proper inoculum-level selection, first the relevance of the inoculum dosage should be considered in relation to the natural populations found in the immediate growing area. In 1972, in Aroostook County, Maine, the average Verticillium population was 85 × 10^3 propagules/g of soil. This could be related to a seed piece inoculum level of 4 × 10^5 spores/ml, as seen in Table 1. The rating for Abnaki at the 4 × 10^5 spore level in 1972
was 2.0 in the inoculum level plot (Table 2). Thus, both
the population that we developed by use of $8 \times 10^4$
spores/ml (Table 1) and our disease rating of 2.8 in the
disease resistance trials (Table 2) are abnormally high.
The 1972 disease index of 3.0, which developed in the
inoculum level plot for Abnaki at $8 \times 10^4$ spores/ml,
places this cultivar in a range with other susceptible lines.

There is still controversy as to whether it is better to
screen at higher inoculum limits and retain only
ultrarresistant materials, or to screen at natural inoculum
levels. If inoculum levels are not standardized in disease
tests throughout the various potato growing regions,
there will be little basis for comparing cultivar reactions in
different locations. The effects of soil and environment
alone would make the results difficult to compare. These
factors would tend to support screening at higher-than
normal inoculum levels, because the ultrarresistant clones
might maintain their resistance under various conditions.
If one is breeding potatoes where resistance to Verticillium
wilt and other diseases and also quality improvement are considerations, the higher inoculum
levels might eliminate, because of inducing susceptible
ratings, some excellent parental materials.

If a disease index of 3.0 or greater is arbitrarily
designated as susceptible, then the inoculum level did not
influence the disease ratings of the early and medium-
maturing cultivars in 1972 (Table 3). This trend did not
continue in 1973 because Monona, Alamo, and Haig were
susceptible when seed pieces were inoculated with $10 \times
10^4$, but not the $2 \times 10^4$ spores/ml. The remaining early
and medium-maturing cultivars were susceptible at low
inoculum levels, a reaction that indicated ultrasusceptibility. With the late-maturing cultivars, the
inoculum level influenced their disease ratings in both
1972 and 1973. The best examples were Abnaki and
Kennebec. Because it is possible to manipulate disease
reaction, we suggest screening at levels more nearly
representative of natural populations. This will provide a
more useful evaluation of clonal reaction in a given
location. If higher levels of resistance are needed at a
location, clones rating 1.0 to 1.5 should be selected and
screened at that location.

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