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

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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.

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Additional key words: population density, clones.

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 8×10^4 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

Spore concentration of seedpiece dip	*Verticillium* population[a,b] (propagules × 10^3/g soil)	
(× 10^4/ml)	1972	1973
0	39 v	22 v
2	62 w	47 w
4	91 x	54 w
6	120 y	96 x
8	118 y	100 x
10	170 z	117 y

[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.

Tolmsoff (5) showed that the inoculum level in the soil is the main factor that determines the rate of disease development. The infection processes occur at the same time, regardless of the inoculum level, but symptoms and death develop more rapidly at the higher inoculum levels. Soil population levels of the pathogen in potato fields have been studied (1, 4), but the microsclerotial form was involved. In Maine, *V. albo-atrum* generally exists in the nonsclerotial form. This study was conducted to determine the proper inoculum level of *V. albo-atrum* suitable for disease testing in Aroostook County, and compare it to natural soil populations.

MATERIALS AND METHODS.—In 1972 and 1973, a split-plot experiment was designed and conducted in Presque Isle to study *Verticillium* development on potatoes in relation to inoculum level. An isolate of *V. albo-atrum,* originating from infected potato roots from Presque Isle, was grown in a shake culture in potato-dextrose broth (250ml/flask) for about 2 weeks. These cultures were pooled and filtered through cheesecloth, and the spores in the filtrate were counted by use of a hemacytometer. The spore suspension was adjusted with distilled water to give final concentrations of 2, 4, 6, 8, and

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

Source of test	Year	Soil population[a] (propagules × 10^3/g)		Disease[b] index		Inoculum level (spores × 10^4/ml)
		Abnaki	Kennebec	Abnaki	Kennebec	
USDA breeding plot	1972	115	110	2.8	3.8	8
	1973	130	129	1.4	4.4	8
Verticillium inoculum-level plot (Table 3)	1972	80	91	2.0	3.0	4
		111	118	3.0	3.5	8
	1973	55	54	1.0	4.0	4
		92	100	2.1	4.2	8
Composite soil samples from Aroostook County	1972	42	85	1.2	2.0	...
	1973	57	63	1.0	2.5	...

[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

Cultivars	Disease reactions following inoculation with:					
	Inoculum levels (spores × 10^4) (1972)			Inoculum levels (spores × 10^4) (1973)		
	2	6	10	2	6	10
Abnaki	1.3 w[a,b]	3.0 x	3.0 x	1.0 v	1.0 v	2.1 x
Houma	2.0 x	3.0 x	3.0 x	1.3 v	1.5 vw	2.5 x
Katahdin	2.5 x	3.0 x	3.0 x	2.5 wx	2.1 wx	3.5 y
Kennebec	2.0 x	3.0 x	4.0 xy	2.3 w	3.5 yz	4.5 z
Monona	3.5 y	4.0 yz	4.5 yz	2.5 wx	3.0 y	3.5 y
N. Russet	3.5 y	3.5 xy	4.0 xy	3.0 xy	3.5 yz	4.0 yz
Superior	4.5 z	4.5 z	5.0 z	3.5 yz	3.5 yz	4.0 yz
Alamo	4.5 z	4.5 z	4.5 yz	2.5 wx	2.3 x	3.5 y
Norland	...	NO TEST	...	3.5 yz	3.5 yz	3.5 y
Cobbler	...	NO TEST	...	4.0 z	4.0 z	4.5 z
Haig	4.5 z	4.0 yz	5.0 z	2.1 w	3.0 y	3.5 y
Sable	4.0 yz	4.5 z	4.5 yz	3.5 yz	4.0 z	4.0 yz

[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 replications, 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 zinc ion maneb (2.25 kg/ha). Plants were rated on a 1-5 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 the plant rhizosphere. The samples were assayed for *Verticillium* populations by use of a soil dilution-plate method (3).

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 (2). 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 $51\text{-}120 \times 10^3$ propagules/g of rhizosphere soil and on 27 August, 1973, from $33\text{-}160 \times 10^3$ 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 10×10^4 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 than in 1973; thus the slow disease development was negated by a dry 3 weeks before harvest. The disease ratings, even at the lowest inoculum level, were higher in 1972 than in 1973. Soil populations also were higher than 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 8×10^4 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 4×10^4 spores/ml, as seen in Table 1. The rating for Abnaki at the 4×10^4 spore level in 1972

was 2.0 in the inoculum level plot (Table 2). Thus, both the population that we developed by use of 8×10^4 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 8×10^4 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 ultraresistant 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 ultraresistant 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 10×10^4, but not the 2×10^4 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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