Use of Quantitative PCR Diagnostics to Identify Tolerance and Resistance to *Verticillium dahliae* in Potato

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ABSTRACT

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A potato breeding population was evaluated for the presence of tolerance and resistance to *Verticillium dahliae*. Clones were ranked as susceptible or moderately and highly resistant based on symptom expression, and a species-specific polymerase chain reaction (PCR)-based assay was used to determine the *Verticillium* biomass in the bases of stems. A clone was designated as tolerant if the amount of fungus present in the host tissue was equal to or greater than the collective average amount for all clones in the symptom category above. Tolerant and resistant clones were identified in all trials, but the expression of tolerance was usually unstable across runs. The fungus was detected in some symptomless plants; on the other hand, some symptomatic plants were found to be pathogen-free. These observations emphasize the necessity of accurate quantification of the pathogen biomass in potato during *Verticillium* resistance breeding programs.

Additional keywords: colonization, *Solanum tuberosum*, vascular wilt

Verticillium wilt caused by *Verticillium dahliae* is a serious vascular disease which annually results in major crop losses wherever potatoes are grown. Several measures are available to control wilt, including breeding for host resistance (24). While this approach is generally the most practical method of disease management, breeding for *Verticillium* resistance in potato (*Solanum tuberosum*) has been less successful than for many other crops (14,25). Most of the commonly grown potato cultivars are susceptible (24). While several factors may have contributed to this problem over the long term, one of the most important probably is the fact that the majority of breeding programs have relied on symptom expression as an indicator of the level of resistance without actual assessment of pathogen biomass in the plant.

Monitoring of *Verticillium* levels may be particularly important with potato. Symptoms are difficult to distinguish from some physiological disorders (24). Potentially, this can result in the diagnosis of false suspects. Also, several studies have found that in certain potato clones or cultivars, some plants may be colonized extensively but develop few symptoms (5,16). Appar-

ently, the absence of symptoms can result from either the limitation of colonization (i.e., the expression of resistance) or the limitation of symptom development in the presence of substantial fungal growth (i.e., the expression of tolerance), but these two conditions cannot be distinguished on the basis of symptoms alone (18).

Lack of quantitative data in past programs is related directly to the absence of a rapid and reliable quantitative assay for vascular pathogen levels. Tests that have been used relied mainly on the plating of plant parts (7) or sap (10,13,14). These methods are semi-quantitative and impractically time-consuming and labor intensive, and thus frequently are avoided. Immunoassay approaches are rapid, accurate, and sensitive but cannot differentiate *V. dahliae* from *V. albo-atrum* (22,23), an important consideration in hosts like potato that can harbor both.

Recently developed polymerase chain reaction (PCR)-based methods for the identification and quantification of plant-pathogenic *Verticillium* species are fast, sensitive, and highly specific (12,19,26). This approach has been used successfully to determine pathogen biomass in *Verticillium*-infected alfalfa, sunflower (11,12), and tomato (8,9). In the present study, PCR-based diagnostics were used to quantify the levels of fungal DNA in a potato breeding population challenged by *V. dahliae*. The objectives were to determine to what extent misdiagnosis based on symptom expression does occur and if tolerance to colonization could be distinguished

satisfactorily using these quantitative approaches.

MATERIALS AND METHODS

Plants. One hundred and twenty-one potato breeding accessions (clones) derived from 37 crosses were used for greenhouse evaluation of wilt disease. Most accessions had a history of resistance or tolerance to *Verticillium* in their ancestry (6), and all were selected because of other desirable agronomic traits. Potato cultivar Kennebec, which is highly susceptible to *V. dahliae*, and two other cultivars with intermediate resistance, Norchip and Russet Burbank, were used as positive controls (24).

After 4 months' storage at 4°C, clones were established from a single eye from seed tubers. Clones comprised 6 plants for greenhouse trials and 10 plants for field trials. In the greenhouse, individual plants were grown in 15-cm (6-in) pots containing pasteurized (65°C for 14 h) soil (Promix, Promix Inc., Rivière du Loup, Quebec) with a 16-h photoperiod. Temperatures fluctuated between 18 and 28°C. Watering, fertilization, and insect control were accomplished by standard practices recommended for the greenhouse. There were three greenhouse trials, designated I, II, and III.

Selected resistant and susceptible clones were field tested subsequently in *Verticillium*-infested experimental plots at the Cambridge Research Station (CRS) of the University of Guelph. The inoculum level of the field was unknown. Cultural practices were as recommended for the area, and irrigation was by sprinkler as needed to maintain optimum water availability.

***V. dahliae* isolates and inoculation.** Seven *V. dahliae* isolates—FIDB, FIID, FIIE, 235, 237, 238, and 240 (J. Robb, private collection)—were collected from commercial potato fields at Alliston and Simcoe, Ontario, and used to produce a mixed inoculum for use in these studies. The individual isolates were classified first based on standard taxonomic criteria (15). The preliminary identification was confirmed by PCR-based diagnostics using species-specific primer sets for *V. dahliae* and *V. albo-atrum* subgroups 1 and 2 (19,27), as well as *V. tricornutum* (17). Stock cultures of the individual isolates were stored as a conidial suspension (4×10^7 conidia per ml) in 25% glycerol at -70°C. To revive stock cultures, single droplets of

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thawed suspension were pipetted directly onto potato dextrose agar (PDA) plates including two antibiotics, kanamycin and ampicillin, each at a final concentration of 50 µg/ml, and the plates were incubated at 26°C in the dark for 4 weeks. Spores were washed from the plates with sterile water, and immediately before inoculation, a spore suspension (5×10^5 per ml of sterile distilled water) was prepared from each isolate according to the method used by Pegg and Street (20). Inocula of the seven isolates were mixed equally to give the final suspension mixture. The aggressiveness of the mixed inoculum was compared with the aggressiveness of *V. dahliae* mixes used in two other *Verticillium* testing programs (H. W. Platt and G. Lazarovits, Agriculture and Agri-Food Canada, *personal communication*). Inocula were tested in the greenhouse on Norchip plants (6) and scored for symptom expression at 63 days postinoculation (dpi).

In greenhouse trials, plants were inoculated at 35 days postplanting (dpp) by root drenching with 100 ml of mixed spore suspension per plant (5). The soil around the root system was cut at four points along the outer edge of the pot with a sterile knife; inoculum was poured into the soil, and 1 day later the plants were watered thoroughly. Negative control plants were treated similarly except that 100 ml of sterile distilled water replaced the inoculum. Three plants of each clone or positive control cultivar were infected with *V. dahliae*, and three were used as uninoculated controls. In the field trial, 10 potato plants of selected clones were started from single eyes planted in a single-row plot approximately 8 m in length using a randomized block design. The in-row spacing was 30 cm and the between-row spacing was 0.9 m. Ten plants each of the cultivars Norchip and Russet Burbank were included as positive controls. At 35 dpp, plants were inoculated with *V. dahliae* using the same method as previously described for the greenhouse trials. There were no negative field controls since the field had a prior history of *Verticillium* wilt.

Sample collection. At 10, 21, 35, and 63 dpi, plants were assessed for the severity of wilt symptoms using a scale of 0 (no symptoms) to 5 (plant dead). A *Verticillium* wilt index (VW) for each clone or cultivar was determined by averaging the disease scores of individual plants. Basal stem sections, 6 cm in length, were collected from control and test plants at the final sampling time, 63 dpi. In the field, these were stored on ice temporarily. All samples were washed in running tap water and then rinsed with 75% ethanol for surface sterilization. After the ethanol had evaporated, the samples were plunged immediately into liquid nitrogen, freeze-dried, and stored at -20°C (6). The stem tissue of all inoculated or all uninoculated plants of a clone or cultivar was pooled for

the quantification of *V. dahliae* DNA in the samples.

DNA/RNA extractions. Nucleic acid was prepared by grinding 0.1 g of freeze-dried tissue (6) in liquid nitrogen using a mortar and pestle, resuspending in 5 ml of sodium dodecyl sulfate (SDS)-extraction buffer (0.3% SDS, 0.14 M NaCl, and 0.05 M Na acetate) and 5 ml of water-saturated phenol (29), followed by homogenization (5 min). The homogenate was incubated for 10 min at 65°C with occasional mixing. The phases were separated by centrifugation at 10,000 rpm (10°C) for 10 min, and 1.5 ml of the aqueous phase was precipitated with 2 volumes of 2% potassium acetate in 95% ethanol. The precipitate was rinsed with 95% ethanol, dissolved in 300 µl of dH₂O, reprecipitated with a ½ volume of 7.5 M NH₄ Ac and 2 volumes of 95% ethanol, and finally resuspended in 300 µl of sterile distilled water. The extracts were stored at -20°C for PCR analysis.

Quantification of fungal biomass. Fungal biomass in each tissue sample was estimated using PCR amplification to determine the amount of fungal DNA in total DNA extracts using *Verticillium*-specific primers directed at the intervening transcribed spacers (ITS1 and ITS2) of the ribosomal genes (17,19). An internal control DNA was included in the reaction to allow for an accurate quantification of the pathogen (12,26). The internal control also served to detect the presence of inhibiting compounds which are present in virtually all potato extracts; adequate sample dilution then was used to minimize this inhibition (26). The PCR amplification was performed using 30 reaction cycles consisting of a 1-min denaturation step at 94°C, a 1-min annealing step at 60°C, and a 2-min elongation at 72°C. PCR amplification was conducted in a volume of 50 µl containing 0.2 mM bovine serum albumin, 0.2 mM of each deoxyribonucleotide triphosphate, 12.5 pmol of each oligonucleotide primer, 1.0 pg of internal control plasmid DNA, 1 unit of *Taq* DNA polymerase, and 7 µl of nucleic acid extract, suitably diluted to minimize the effect of inhibitors. PCR products were fractionated on a 2% agarose gel and stained with ethidium bromide solution (25 µg/ml) for 35 min. The fungal and internal control bands were scanned using the Bio-Rad image system "Gel Doc 1000" (BioRad Laboratories Ltd., Hercules, CA). The fungal band-internal control band ratio was calculated, and the amount of *V. dahliae* DNA was determined from a standard curve (6,12). The data for *V. dahliae* DNA content in potato stem samples were not distributed normally; therefore, the analysis of variance (ANOVA) and correlation coefficient were determined from log transformed ($1 + \text{ng } V. dahliae \text{ DNA/g fresh tissue}$) values with the SigmaStat (Jandel Scientific, San Rafael, CA) program. All fungal DNA levels cited in

this paper are expressed in log ($1 + \text{ng } V. dahliae \text{ DNA/g fresh tissue}$).

Determination of tolerance. In each trial, individual clones or cultivars were classified into three categories based on symptom expression. If the wilt index was equal to or less than 2.5, the clone or cultivar was classified as low (L) with respect to symptom expression; between 2.6 and 3.7 it was classified as moderate (M); and equal to or greater than 3.8 it was classified as high (H). For trials II and III, the collective average wilt index also was determined for each category at 63 dpi.

In each trial, individual clones or cultivars also were classified into three categories based on the average amount of fungal DNA in the lower stem samples of infected plants. If the fungal biomass, measured as log ($1 + \text{ng } V. dahliae \text{ DNA/g fresh tissue}$), was equal to or less than 1.0 ng, the clone or cultivar was classified as low (L) with respect to fungal biomass; between 1.1 and 2.3 it was classified as moderate (M); and equal to or higher than 2.4 it was classified as high (H).

Both the wilt index and the fungal biomass were considered in the final ranking of the interaction between each clone or cultivar and *V. dahliae*. Those interactions in which both parameters were classified as low were ranked as resistant (R), while those in which both parameters were moderate or high were ranked as moderately resistant (M) or susceptible (S), respectively. In any trial, clones or cultivars were classified as tolerant (T) if the average fungal biomass was equal to or greater than the collective average *Verticillium* DNA of the symptom category above for that trial; hence, some clones or cultivars with low symptom expression were ranked as tolerant (R/T), as well as some with moderate symptom expression (M/T).

RESULTS

Inoculum. PCR-based diagnostics showed that all isolates included in the inoculum mix were positive for *V. dahliae* alone (i.e., results not shown), as expected, since this is the prevalent species found in potato in southern Ontario (15). When the aggressiveness of the mix was compared with the aggressiveness of mixes used in two other *Verticillium* testing programs, the Guelph wilt index on Norchip plants at 63 dpi, 3.5 ± 0.6 , was midway between the wilt indices produced by the other two mixes, 2.6 ± 0.6 and 4.3 ± 0.7 .

Symptoms. Symptoms first became distinguishable between 12 and 14 dpi in greenhouse and field trials. Typical symptoms (Fig. 1) included chlorosis and necrosis of the leaves, leaf flaccidity, stunting, and in extreme cases, yellowing of the stem and plant death. Although there were differences among trials, within a trial the infected plants of a clone had similar scores.

Greenhouse trials. In the three greenhouse trials, clones were classified into

three different categories based on their wilt indices: low, moderate, and high. When the first trial, which was considered preliminary, was assessed and terminated at 35 dpi, wilt indices ranged from 0 to 4.0. When the population was divided into three categories of roughly equal size, the wilt indices of the least symptomatic plants averaged 0.6, the intermediate group averaged 2.3, and the highest group averaged 3.6 (6). When the same clones were retested, the corresponding averages at 35 dpi were 1.4 (L), 2.3 (M), and 3.3 (H), and at 65 dpi were 2.1 (L), 2.9 (M), and 4.0 (H).

Based on their performance in the first two trials, 65 clones were assessed by PCR at the end of trial II (Table 1). These com-

prised the 38 least symptomatic plants, the 9 with the most symptoms and the 18 that gave variable responses but showed low disease levels in one of the two trials. These clones now were ranked as resistant, moderate, susceptible, or tolerant. The average amounts of fungal DNA and average wilt indices for the categories of tested plants are given in Table 1. There was a linear correlation between the wilt indices and average amounts of fungal DNA in the lower stems for the 65 clones as determined by both Pearson's ($r = 0.66$, critical value [CV] = 0.24), and Spearman's ($r_s = 0.63$, CV = 0.36) correlation analyses at $P < 0.001$. Some clones were lost after trial II because of insufficient tuberization, but 52 clones were retested in trial III. These

included 21 plants with some resistance, 13 that gave susceptible results, and 19 that gave variable responses. These results also are summarized in Table 1. Pearson's product moment analysis ($r = 0.28$, $P = 0.05$) again indicated a correlation between VW scores and fungal DNA levels, but Spearman's rank order analysis did not ($r_s = 0.22$, $P = 0.11$). When the disease scores of the three trials were compared, the scores were highest in trial III. We also found that tolerance was unstable and a major contributor to the "uncertain" category. In the second trial, seven clones were found to have higher fungal biomass than expected based on symptom scores, and these were classified as tolerant, but all were susceptible in the third trial. Seven different clones also were scored as tolerant in the third trial; previously these had been scored as resistant, moderately resistant, or susceptible.

Throughout the three trials, 13 clones showed low or moderate wilt scores, below 3.7. None of the 13 were tolerant in trial II, but four were scored as tolerant in trial III (Fig. 2). All also had other desirable traits, for example, satisfactory tuber yield and short sprouting time. These were tested further for resistance or tolerance in the field.

During the three trials, a total of 81 control plants developed wiltlike symptoms. All members of a clone were not affected similarly. Each affected plant was assayed for the presence of *V. dahliae*, and 10 symptomless uninoculated plants also were tested. Ninety-six percent of the symptomatic plants were free of detectable *V. dahliae*; however, one clone in the S category was colonized extensively with a DNA level even higher than the average DNA level in infected susceptible plants (Table 2).

Field trial. The 13 clones that consistently were found to have low or moderate wilt scores were planted in a field with a history of Verticillium wilt. The field trial also included three clones that continued to give ambiguous results: 02-16, 08-20, and 22-2. A moderately resistant cultivar, Russet Burbank, a susceptible cultivar, Kennebec, and two reference clones (37-1, 37-12) were included as susceptible controls (Table 3). Ten clones of the 16 tested were found to express resistance or tolerance. Among those ranked as tolerant, one had a wilt index in the low category and two had wilt indices classified as moderate.

DISCUSSION

While the phenomenon of "tolerance" has been recognized in plant pathology for over a century (4), the word has been defined in various ways, leading to considerable confusion in its usage (2,3,18). With respect to vascular wilts, some have equated the term with "symptomless carriers" (21) and others with "intermediate resistance" (1,28), but these are very dif-

Fig. 1. Verticillium wilt (VW) scores for assessing symptoms in *Verticillium dahliae*-infected potato plants. From left to right: 0, no symptoms; 1, 1 to 12% involvement; 2, 13 to 25% involvement; 3, 26 to 50% involvement; 4, 51 to 75% involvement; 5, severe wilting involving entire plant to death.

Table 1. Number of potato clones in each symptom expression category evaluated for the average amount of *Verticillium dahliae* DNA and the average wilt index at 63 days postinoculation in trials II and III

Trial	Category	Clones (no.)	Average <i>V. dahliae</i> DNA level ^a	Average wilt index ^b
II	L ^c	16	0.7	1.9
	M	22	1.6	2.8
	U	18	2.8	4.2
	H	9	4.9	4.4
		65	2.3	3.6
III	L	6	2.2	3.0
	M	15	3.0	3.3
	U	19	3.3	4.8
	H	13	3.5	4.9
		53	3.2	4.3

^a Average *V. dahliae* DNA level: log (1 + ng *V. dahliae* DNA/g fresh tissue).

^b Plants were scored for disease symptoms based on a 0 (no disease) to 5 (plant dead) point scale. The wilt index is the average wilt score/clone.

^c Categories are based on Verticillium wilt (VW) scores: (L) low, ≤ 2.5 ; (M) moderate, 2.6 to 3.7; (H) high, ≥ 3.8 ; (U) uncertain, VW score variable from experiment to experiment.

ferent concepts. In this context, we prefer the definition of Clarke (3), who states that "tolerance of the parasite would be the ability of a plant to endure the effects of levels of parasite infection which, if they occurred at equivalent levels in other plants of the same or similar species would cause greater damage or disease." Two aspects of this definition are particularly important: the requirement for quantification (i.e., levels of pathogen, amount of disease) and the requirement for comparison among infected plants.

Clearly, tolerance is a quantitative characteristic; yet the literature provides little guidance on methods of assessment for vascular wilt diseases. Most attempts to determine pathogen levels have been cursory. Based on cytological data, Beckman and Roberts (1) devised a "colonization quotient" to monitor rates of colonization, but this approach is unsuitable for PCR-quantification. Lynch et al. (14) and Corsini et al. (5) distinguished resistant (R) and tolerant (T) plants on an arbitrary basis. In the second study (5), both resistant and tolerant plants had "few" or no symptoms, but an R rating indicated colonization was very low (7), whereas a T-rating indicated colonization was similar to that in susceptible plants. The difficulty with the latter approach is that, in our experience, the range in levels of fungal DNA found in susceptible reactions can be very large (Table 3). In the present study, all clones or cultivars were classified into three categories based on symptom expression and three categories based on the average amount of *Verticillium* DNA in the lower stems. Further adapting Corsini and Pavék's approach to Clarke's definition, we have designated a clone as "tolerant" if the amount of pathogen DNA was equal to or greater than the collective average amount for all clones in the symptom category above. Hence, a clone would be labeled tolerant if the wilt index ≤ 2.5 but the amount of fungal DNA present was more typical of the moderate category. A resistant clone could have a wilt index ≤ 2.5 but would have less fungal DNA present. Also, a clone could be designated tolerant if the wilt index was between 2.6 and 3.7 but the DNA level was more typical of the susceptible category. In this study, approximately 12% of the clones were scored as tolerant in each of the greenhouse trials, although the frequency was somewhat higher in the field test, which was more intensively selected; of the 16 potentially resistant clones, three were tolerant and seven resistant. Hence, the accurate diagnosis of tolerance seems a significant problem in breeding programs, especially as the condition appears to be unstable. From trial to trial, some clones remained in the same symptom category but the amount of fungus in the tissues differed. However, other clones that were ranked as tolerant in one or

more trials were classified in a different symptom category in other trials.

In general, the reported variability in colonization levels within a clone among greenhouse trials and between greenhouse and field trials could represent another hazard in breeding selection. In our experience studying the *Verticillium* interaction with potato and other host species, the most likely sources of variation lie in the inoculation process itself and in environmental variables such as temperature, day length, and light levels. While many studies have addressed the effect of these parameters on symptom expression in a variety of species, few have adequately documented *Verticillium* colonization because of the lack of efficient, reliable methods of quantification. The molecu-

lar diagnostic techniques used in the present paper should provide a useful tool to determine which are the most important factors giving rise to the variable colonization levels of some clones in successive trials and to monitor the effectiveness of possible approaches to controlling these factors in future programs.

During this study, 81 control plants also developed wiltlike symptoms, but only four tested positive for *V. dahliae*; *V. albo-atrum*, another common pathogen in potato, was not detected. The source of the contamination is unknown. The most likely causes of the symptoms developing in control plants were natural senescence and drought stress, both of which can mimic wilt symptoms in potato (24).

Fig. 2. Polymerase chain reaction (PCR) products of the DNA extracts (trial III) from the 18 clones selected for field trials. DNA was extracted from 0.1 g of freeze-dried basal tissues and used for PCR amplification. Resulting PCR products were fractionated on a 2% agarose gel. *Verticillium dahliae* DNA was quantified for each clone by determining the average of two PCR reactions. The code above each lane specifies one of the clones listed in Table 3 or 1.0 pg internal control (ctl). The *Verticillium* wilt index (VW) and the average DNA level for each clone are given below the lanes.

Table 2. Total number of uninoculated control plants, with and without wilt-like symptoms, evaluated for the amount of *Verticillium dahliae* DNA in lower potato stems at 63 days postinoculation in trials I to III

Category	Plants assessed	Average <i>V. dahliae</i> DNA level ^a		
		0	0.1 to 3	>3
With wiltlike symptoms	81	77	3	1
Without wiltlike symptoms	10	10	0	0
Subtotal	91	87	3	1

^a Average *V. dahliae* DNA level: $\log (1 + \text{ng } V. \text{dahliae DNA/g fresh tissue})$.

Table 3. Characteristics of 16 potato clones selected for the field trial, summarizing their ancestry as well as wilt indices and average amount of *Verticillium dahliae* DNA in lower stems at 63 days postinoculation in trials II and III and in the field^a

Clone or cultivar	Parentage ^b	Trial II			Trial III			Field trial		
		VW ^c	DNA ^d	Rank ^e	VW	DNA	Rank	VW	DNA	Rank
01-1	R×R	3.0(M) ^f	0.0(L) ^g	M	2.2(L)	3.8(H)	R/T	0.9(L)	0.0(L)	R
02-7	T×?	3.0(M)	0.0(L)	M	3.0(M)	3.7(H)	M/T	3.8(H)	2.1(M)	S
02-16 ^{*h}	T×?	4.3(H)	4.4(H)	S	2.8(M)	2.7(H)	M	2.1(L)	0.7(L)	R
06-8	R×R	2.8(M)	0.0(L)	M	3.3(M)	3.1(H)	M	1.9(L)	0.0(L)	R
06-19	R×R	1.7(L)	0.0(L)	R	3.0(M)	2.4(H)	M	3.2(M)	1.0(L)	M
07-5	R×R	1.2(L)	0.0(L)	R	3.3(M)	3.1(H)	M	2.2(L)	0.0(L)	R
07-27	R×R	2.8(M)	2.9(H)	M	3.5(M)	2.1(M)	M	2.5(L)	0.0(L)	R
07-44	R×R	1.0(L)	0.0(L)	R	3.7(M)	3.1(H)	M	4.2(H)	2.1(M)	S
08-20 [*]	T×?	4.7(H)	0.8(L)	S	3.2(M)	2.3(M)	M	2.4(L)	0.0(L)	R
09-28	T×?	3.0(M)	1.0(L)	M	3.2(M)	3.2(H)	M	3.7(M)	0.0(L)	M
09-29	T×?	2.8(M)	1.9(M)	M	3.7(M)	1.7(M)	M	2.4(L)	0.0(L)	R
104PY	?×?	2.7(M)	3.1(H)	M	2.5(L)	3.4(H)	R/T	1.8(L)	1.6(M)	R/T
26-6	T×?	2.3(L)	0.0(L)	R	3.3(M)	3.2(H)	M	3.9(H)	2.4(H)	S
36-7	?×?	2.3(L)	0.0(L)	R	2.3(L)	1.0(L)	R	3.4(M)	1.6(M)	M/T
F18R	SM ^z	1.8(L)	0.0(L)	R	2.5(L)	3.3(H)	R/T	3.4(M)	2.6(H)	M/T
22-2 [*]	?×?	3.3(M)	2.6(H)	M	5.0(H)	3.8(H)	S	4.9(H)	0.0(L)	S
37-1	?×R	5.0(H)	5.0(H)	S	5.0(H)	4.0(H)	S	4.3(H)	1.0(L)	S
37-12	?×R	4.7(H)	4.4(H)	S	4.5(H)	4.5(H)	S	4.2(H)	2.6(H)	S
Norchip	?×?	2.5(L)	2.7(H)	S	3.7(M)	2.1(M)	M	— ⁱ	—	—
Kennebec	S×S	—	—	—	—	—	—	4.3(H)	1.8(M)	S
Russet Burbank	—	—	—	—	—	—	—	3.3(M)	0.0(H)	M

^a Each clone is ranked, relative to all others in the trial, based on symptoms and fungal biomass present.

^b Note that the resistant and tolerant rankings of ancestral plants were not determined by the methods used in this paper.

^c VW = Verticillium wilt index, average disease score/clone based on a 5-point scale (0, no symptoms; 5, plant dead).

^d DNA = average amount of *V. dahliae* DNA/clone assessed by PCR units: log (1+V. *dahliae* DNA ng/g fresh tissue).

^e Rank = collective ranking of clone based on assessment of the VW and fungal DNA: R, resistant; M, moderate; S, susceptible; T, tolerant.

^f VW scores were divided into three categories in all trials: L, ≤2.5; M, 2.6 to 3.7; H, ≥3.8.

^g DNA levels were divided into three categories in all trials: L, ≤ 1.0; M, 1.1 to 2.3; H, ≥2.4.

^h * = clones with variable results in greenhouse trials (I to III) but which were in the low category in at least one trial.

ⁱ — = no data available.

These results further support the contention that symptom expression alone is not a reliable indicator of levels of *Verticillium* sp. associated with wilt disorders in this host. Without adequate quantification of the fungus, misdiagnosis is a real hazard for breeding programs. However, the quantitative approaches used in this study are an efficient method of detecting and minimizing the problem and of identifying tolerant plants in the population.

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