Stability of Resistance of Potato to Strains of *Verticillium dahliae* from Different Vegetative Compatibility Groups

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ABSTRACT

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Potato (*Solanum tuberosum*) clone A66107-51 was resistant to a range of potato strains of *Verticillium dahliae*. These included strains from three vegetative compatibility groups, P1, P3, and P4. Resistance was determined by symptom expression and colonization of stems under greenhouse conditions. The wilt-susceptible cultivar Russet Burbank was very susceptible to strains from compatibility group P4, moderately susceptible to a strain from group P3, and not susceptible to a strain from group P1 and an unclassified strain. The Verticillium wilt resistance of clone A66107-51 was not affected by the vegetative compatibility group or geographic origin of *V. dahliae* strains.

The development of Verticillium wilt-resistant parental germ plasm and cultivars has been a major objective of the USDA, ARS, potato-breeding program at Aberdeen, ID. As a result of this emphasis, Verticillium wilt-resistant clones with other agronomically important characteristics have been selected and used extensively as parents (1).

Several of these parental potato clones have shown a high degree of stability in field resistance to Verticillium wilt (3); however, loss of disease resistance is a common phenomenon in many crops. For example, the field resistance of the Acala cotton cultivars to Verticillium wilt was overcome by a more virulent pathotype of *V. dahliae* (6). The more virulent pathotype was found by heterokaryon incompatibility tests (5) to belong to a different genetic group.

Because of the potential for loss of resistance, potato genotypes with different degrees of susceptibility to *V. dahliae* were tested for resistance to five *V. dahliae* strains. These strains belonged to three vegetative compatibility groups from widely different geographic areas.

MATERIALS AND METHODS

Potato isolates of *V. dahliae* that had been classified by compatibility group were obtained from A. A. Bell of the National Cotton Pathology Research Laboratory, College Station, TX. Compatibility classification was determined by Puhalla (4,5) and was based on the ability of isolates within a vegetative compatibility group to form heterokaryons with one another but not with those in other groups.

All *V. dahliae* isolates were tested for virulence on eggplant cultivar Black Beauty. Five eggplant seedlings were transplanted into 10-cm pots containing standard greenhouse mix to which microsclerotia of *V. dahliae* inoculum grown on oats was added (2 g/pot). Wilt readings were made 32 days after transplanting, and stem sections were assayed for *V. dahliae* colonization by the method of Davis et al (2).

In the first experiment, rooted cuttings of four potato clones, A68113-4, A66107-51, Russet Burbank, and Butte, were inoculated by dipping roots into a conidial and microsclerotial suspension adjusted to 10^8 colony-forming units (cfu) per milliliter of each isolate. These were immediately planted in pathogen-free greenhouse soil mix (one part peat, two parts washed sand, and two parts vermiculite) in 15-cm pots and arranged on greenhouse benches in a randomized complete block design.

In the second experiment, eyes were removed from surface-disinfested (0.5% sodium hypochlorite) tubers with a melon ball scoop to obtain uniform potato plants for greenhouse testing. These were sprouted on a moist peat medium. The sprouted eyes were inoculated and planted when the sprouts were about 3–4 cm long and the roots were 3–6 cm long. Inoculum was prepared from a combination of 30-day-old potato-dextrose agar (PDA) plates with heavy microsclerotial production and 2-wk-old PDA plates with heavy microconidial production. Inoculum concentration for all strains was between 10^8 and 10^9 cfu/ml. The sprouted eyes and roots were dipped for 5 sec in inoculum and immediately planted in pathogen-free greenhouse soil mix in 15-cm pots. The pots were placed on greenhouse benches in a randomized complete block design consisting of four replicates (three pots per treatment). During these experiments, the greenhouse temperatures fluctuated between 18 and 27 C with intermediate day length conditions (14 hr of daylight).

Symptoms of Verticillium wilt were assessed on a scale of 0–5, where 0 = no symptoms; 1 = trace of chlorosis or necrosis in lower leaflets with no wilting; 2 = lower leaflets chlorotic with some necrosis, moderate wilting of some petioles; 3 = 25–50% of petioles showing significant chlorosis/necrosis and definite wilting but stem not yellowing; 4 = 50–90% of petioles showing significant chlorosis/necrosis and wilting with some stem yellowing; 5 = stem greater than 90% dead or dying. Each plant was rated separately and mean values are reported.

Lower leaflet chlorosis (wilt index rating 1) may be an early symptom of wilt

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Table 1. Verticillium wilt resistance of potato breeding clone A66107-51 compared with standard cultivars

Clone	Field wilt index[z]	Greenhouse wilt index[z]	Log cfu of *V. dahliae* per gram of stem
A66107-51	0.6 c	1.5 c	0.00 c
Katahdin	1.1 c	1.6 c	0.65 bc
Russet Burbank	2.7 b	2.4 b	2.81 a
Norgold Russet	5.0 a	3.0 a	2.07 ab

[z] Wilt index on a scale of 0–5, where 0 = no symptoms; 1 = trace of chlorosis or necrosis in lower leaflets with no wilting; 2 = lower leaflets chlorotic with some necrosis, moderate wilting of some petioles; 3 = 25–50% of petioles showing significant chlorosis/necrosis and definite wilting but stem not yellowing; 4 = 50–90% of petioles showing significant chlorosis/necrosis and wilting with some stem yellowing; and 5 = stem greater than 90% dead or dying. Ratings were made 103 days after planting in the field and 70 days after planting in the greenhouse.

or a sign of normal senescence and is commonly seen in both resistant and susceptible plants. Stem colonization was measured along with wilt symptom development because of the possibility of confusing normal senescence with Verticillium wilt and to distinguish tolerance from true resistance. The extent of colonization was determined by the Anderson sampler method described by Davis et al (2). Stem sections 5 cm long were taken 5 cm above the soil line, surface-disinfested with 0.5% sodium hypochlorite, and air-dried. These were assayed within 90 days of sampling.

RESULTS

The high degree of Verticillium wilt resistance exhibited by clone A66107-51 remained stable when tested under controlled conditions against *V. dahliae* isolates from different vegetative compatibility groups and from different geographic areas. In contrast, cultivar Russet Burbank was very susceptible to strains within vegetative compatibility group P4. The strain from group P3 was weakly pathogenic in Russet Burbank, and the strain from group P1 and the nonreactor strain did not cause wilt symptoms or colonize lower stems.

The severity of wilt symptoms caused by *V. dahliae* and the extent of colonization of stem tissue of A66107-51 compared with several standard cultivars are shown in Table 1. The resistance of A66107-51 has remained stable over 8 yr of field testing under different conditions in several locations (2,3). Symptoms of Verticillium wilt have consistently remained negligible during the 120-day growing season in soils heavily infested with the pathogen (>100 cfu/g of soil).

The isolates of *V. dahliae* used in this study are described in Table 2. These were tested for virulence on eggplant cultivar Black Beauty, and all were highly virulent (Table 3). Wilt symptoms on eggplant caused by isolate 207 were significantly less ($P = 0.05$) after 32 days than those caused by the other isolates, but stem colonization was equal.

Results of the first experiment indicated isolates from compatibility group P4 were highly virulent in potato, whereas the other isolates were not. The overall wilt index ratings for four clones 30 days after inoculation were as follows: 0.3 for the uninoculated control, 0.4 for strain T9 (group P1), 0.6 for strain PR (nonreactor), 0.9 for strain 207 (group P3), and 3.2 for strain PW and 3.6 for strain BB (both group P4). Inoculation by dipping cut roots in a high concentration of inoculum was a severe test, but the resistant clones A66107-51 and A68113-4 showed fewer wilt symptoms with isolates from group P4 than did the more susceptible clones (Russet Burbank and Butte). Isolate 207 (group P3) was weakly pathogenic to Russet Burbank under these conditions (wilt index rating 1.8) but did not produce symptoms on resistant clones A66107-51 and A68113-4.

The results of the second experiment confirmed the initial results. *V. dahliae* isolates from compatibility group P4 (BB and PW) were significantly ($P = 0.05$) more virulent in Russet Burbank than isolate 207 in group P3 (Table 4). The isolate from group P1 (cotton-defoliating strain T9), and the nonreactor (PR) did not induce wilt symptoms and did not colonize lower stem tissue. After 58 days, all Russet Burbank plants inoculated with group P4 isolates were showing some degree of wilt symptoms (wilt index rating 2–5). Plants inoculated with the other isolates showed only lower leaf chlorosis (similar to control plants, as a result of normal maturity).

Determinations of the extent of stem tissue colonization indicated that both *V. dahliae* isolates from group P4 were aggressive colonizers. *V. dahliae* stem colonization was highly correlated with wilt symptoms ($r^2 = 0.75$, $P = 0.01$, $n = 32$). Group P3 isolate 207 colonized stems of Russet Burbank plants to a limited extent and was less virulent. In contrast, parental clone A66107-51 was highly resistant to colonization by all *V. dahliae* strains used (Table 5). Stem colonization measurements confirmed the fact that lack of wilt symptoms was due to true resistance.

DISCUSSION

This study has provided evidence that the high degree of field resistance to Verticillium wilt developed by the potato-breeding program at Aberdeen, ID, is not

Table 2. *Verticillium dahliae* strains, vegetative compatibility group, and geographic origin

V. dahliae strain	Compatibility group[a]	Host	Geographic origin
T9	P1	Cotton	California
207	P3	Potato	Australia
BB	P4	Potato	Idaho
PW	P4	Potato	Wisconsin
PR	Nonreactor	Potato	Russia

[a] Puhalla (4).

Table 3. Virulence of *Verticillium dahliae* strains on eggplant cultivar Black Beauty

V. dahliae strain	Wilt index[a] (eggplant)	Log cfu of *V. dahliae* per gram of stem[b]
T9	4.4	4.18
207	3.2[c]	4.31
BB	4.8	4.20
PW	4.6	3.79
PR	4.4	4.25
Control	1.2	0

[a] Wilt index on a scale of 0–5, where 0 = no symptoms; 1 = trace of chlorosis or necrosis in lower leaflets with no wilting; 2 = lower leaflets chlorotic with some necrosis, moderate wilting of some petioles; 3 = 25–50% of petioles showing significant chlorosis/necrosis and definite wilting but stem not yellowing; 4 = 50–90% of petioles showing significant chlorosis/necrosis and wilting with some stem yellowing; and 5 = stem greater than 90% dead or dying.
[b] Composite sample from five plants.
[c] Significantly less wilt than other strains ($P = 0.05$).

Table 4. Reaction of wilt-susceptible potato cultivar Russet Burbank to strains of *Verticillium dahliae* from different vegetative compatibility groups and geographic areas

V. dahliae strain	Compatibility group[z]	Wilt index (58 days)	Log cfu of *V. dahliae* per gram of stem
Control	···	1.0 b	0.00 c
PR	Nonreactor	0.9 b	0.00 c
T9	P1	1.2 b	0.00 c
207	P3	1.5 b	1.24 b
BB	P4	2.8 a	2.68 a
PW	P4	3.2 a	3.14 a

[z] Puhalla (4).

Table 5. Reaction of wilt-resistant potato breeding clone A66107-51 to strains of *Verticillium dahliae* from different vegetative compatibility groups and geographic areas

V. dahliae strain	Compatibility group[z]	Wilt index (58 days)	Log cfu of *V. dahliae* per gram of stem
Control	···	0.8 a	0.00 a
PR	Nonreactor	0.6 a	0.00 a
T9	P1	0.8 a	0.00 a
207	P3	0.9 a	0.00 a
BB	P4	0.8 a	0.40 a
PW	P4	1.0 a	0.40 a

[z] Puhalla (4).

limited to local strains or to certain pathotypes of *V. dahliae*. It also provides support for the concept of localized *V. dahliae* populations with distinctly different host ranges and virulence potential as suggested by Puhalla and Hummel (5). Since the development of Verticillium wilt resistance in potato is of worldwide concern, it is important that clones are tested for resistance to the most virulent potato isolates. Our results indicate that isolates within vegetative compatibility group P4 are the most pathogenic to potato. For future studies, it would be of interest to determine if potato clones that are more susceptible than Russet Burbank can be more aggressively colonized by isolates of *V. dahliae* not belonging to compatibility group P4.

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