Influence of Continuous Cropping of Several Potato Clones on the Epidemiology of Verticillium Wilt of Potato

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ABSTRACT

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Two field studies with potato were conducted to study the effects of continuous cropping of Verticillium-resistant potato clones on the epidemiology of Verticillium wilt of potato. Investigations focused on effects of continuous cropping of different clones on wilt incidence, *Verticillium dahliae* reproduction within the host, changes in the soilborne inoculum density, crop yields, and effects on succeeding crops of susceptible cultivars. Each study involved 5 yr of continuous cropping with potato. Five cultivars (and/or clones) with different levels of resistance to Verticillium wilt were planted in the first study. These were Russet Burbank and Butte (susceptible), Targhee (resistant), and A66107-51 (-51) and A68113-4 (highly resistant). The second study included the susceptible cultivar Russet Burbank, the resistant clone -51, a nonhost (corn), and a fallow treatment. There was no evidence for change in the relative resistance of the cultivars over the course of these studies, nor was there evidence that an extremely virulent strain of *V. dahliae* was enhanced selectively from the indigenous population. All resistant clones remained nearly symptomless and resistant to stem colonization throughout our investigations. The first study, conducted from 1977 to 1982, demonstrated that *V. dahliae* inoculum densities in the soil were negatively correlated with the degree of resistance of the cultivar grown the previous year. Where highly resistant cultivars were grown for five seasons, inoculum densities of *V. dahliae* were 60–70% lower than in plots where susceptible cultivars were grown. The second study, conducted from 1983 to 1988, confirmed that increases in *V. dahliae* inoculum densities were related to cultivar susceptibility. Where the resistant -51 clone was grown, the increase of *V. dahliae* populations was delayed by 1 yr, and populations increased to only 60% of those which developed with the susceptible Russet Burbank. Although *V. dahliae* inoculum densities increased with the cropping of potato clones, no changes in inoculum density occurred in either corn or fallow plots. Inoculum densities in plots with the highly resistant clones decreased to the point that neither the susceptible Russet Burbank nor a highly susceptible potato clone (NDA8694-3) exhibited substantial Verticillium wilt in a subsequent cropping season. When resistant potato clones are integrated into a potato production program, they appear to limit Verticillium wilt severity both during the years in which they are grown and in following crops of susceptible cultivars.

Additional keywords: ecology, *Verticillium albo-atrum*.

Verticillium wilt of potato (*Solanum tuberosum* L.) may be caused by either *Verticillium dahliae* Kleb. or *V. albo-atrum* Reinke & Berth. In Idaho and other arid regions, this disease is caused by *V. dahliae* (9). Verticillium wilt is a major factor limiting the production of the commonly grown cultivars in the United States, including the most important variety, Russet Burbank. Much effort has been placed on developing potato clones with increased Verticillium wilt resistance.

In potato breeding programs, the incidence and/or severity of wilt symptoms and the relative degree of stem colonization by *V. dahliae* (11) must be determined if resistance is to be evaluated reliably. Based on *V. dahliae* colonization in stem tissue, two potato clones, A66107-51 (-51) and A68113-4 (-4) have been shown to be highly resistant to Verticillium wilt compared to Russet Burbank and other very susceptible cultivars (11). These two clones have been resistant to *V. dahliae* in many field trials throughout the Pacific Northwest and the Midwest (*unpublished data*). Other field studies (18) have compared these clones with many potato varieties over several years, and the clones were among the most resistant and the highest yielding. These selections appear to meet the rigid standards of durable resistance (16). Clone -51 is also resistant (7) to several *V. dahliae* pathotypes (21). Immunity to *V. dahliae* colonization in stems has not been identified in tuber-bearing *Solanum* spp., however (8). Stems of resistant clones are colonized by *V. dahliae* but not as extensively as the stems of susceptible cultivars (e.g., Russet Burbank, Kennebec, and Norgold) (11,18).

The performance of these varieties in Verticillium-infested soil after continuous cropping has not been determined. Prudent use of resistant potato clones should involve an understanding of the potential effects of these clones on the epidemiology of Verticillium wilt. A wilt pathogen can colonize roots and contribute to the inoculum in the soil even without causing disease symptoms (15), so an apparently resistant potato clone may not necessarily be an advantage with repeated croppings. In fact, adverse effects might occur with the continuous cropping of resistant clones. Isolates of *V. dahliae* from field-grown plants of tolerant cotton cultivars may be more virulent than isolates from susceptible cultivars, which suggests that tolerant cultivars may select for a particularly aggressive strain of the pathogen (3). Field studies (4) have also demonstrated an increase in disease severity after 5 yr of continuous cropping with the Verticillium-tolerant Acala SJ-5. The percentage of plants that were defoliated increased even though there was a slight but significant reduction of *V. dahliae* inoculum density with continuous cropping.

The objective of this investigation was to evaluate the effect of continuous cropping with Verticillium wilt–resistant potato cultivars and clones on soil inoculum densities of *V. dahliae,* development of Verticillium wilt in subsequent potato crops, and yield. Some data from these studies have been published (10–12).

MATERIALS AND METHODS

Field study 1. The field used for this investigation was located at the University of Idaho Research and Extension Center, Aberdeen, and has a Declo loam soil type. For more than 30 yr prior to this study, the field had been cropped with barley, wheat, and potato, and had been out of potato production during the previous 2 yr. This field had a history of Verticillium wilt on potato crops, with high initial inoculum levels approximating 90–150 colony-forming units (cfu)/g of air-dried soil. Resistant potato clones -51, -4, and Targhee were compared with cultivars Russet Burbank and Butte by continuously cropping each clone for five consecutive growing seasons (1977–1981) in the same plots. During the sixth year of this study, Russet Burbank was grown over all plots as a bioassay of the results. Potato cultivars and breeding clones were arranged in a randomized block design with four replications each. Plot dimensions were 7.2 m wide × 22.5 m long with eight rows per plot and 0.9-m spacing between rows. To inhibit the movement of soil among plots, they were separated laterally by fallow strips 5.2 m wide. Data were collected from the center 7.6 m of the center two rows.

During the 6-yr duration of this study, recommended practices of fertilization, irrigation, and pest control were followed (5,20,22). Each year, preplant applications of NH_4NO_3 and triple superphosphate were applied at 112 kg/ha of nitrogen and 89 kg/ha as P_2O_5. Furrow irrigation was used. Additional N was added as needed (determined with petiole assays) with a single application of urea–ammonium nitrate solution (32% N) dripped into furrows during the growing season at a rate of 112 kg N/ha. Disulfoton (3.4 kg a.i./ha) was side-dressed for insect control at planting. Both EPTC (Eptam, 3.4 kg a.i./ha) and trifluralin (0.56 kg a.i./ha) were applied and disked into the soil prior to planting for weed control.

All seed potatoes used in these studies were produced at or adjacent to the University of Idaho Research and Extension Center, Tetonia, and met certification standards (2). Yearly assays from tuber stem-ends and the planting of seed tubers in the greenhouse in pathogen-free soil provided no evidence for contamination of tubers with *V. dahliae.*

Seed potatoes of field study 1 were cut to 56–84 g and treated with 0.5% captan dust at 1 kg/100 kg of seed. Seed tubers were planted each year between 20 and 23 May with an assist-feed planter.

Field study 2. This study was also conducted at the Aberdeen Research and Extension Center, but in a field cropped to various grasses but not potatoes for more than 30 yr. In contrast to field study 1, the initial soil ID of *V. dahliae* was low (4–8 cfu/g of air-dried soil). Treatments during 1983–1987 included fallow plots and plots cropped with the highly resistant -51 clone, Russet Burbank, or a nonhost grain crop (*Zea mays* L. subsp. *mays* 'Jubilee'). Stem biomasses of Russet Burbank and -51 potatoes were compared by removing plants from 1.5 m of row from each plot on 25 August 1987 and oven-drying and weighing them.

Field plots (7.3 × 18.3 m) were first planted in a Latin square design with four replicates per treatment in 1983, and these same crops (or fallow) were planted in the same respective plots during 1983–1987. In 1988, half of each plot (four rows) was planted to Russet Burbank and the other half to the highly susceptible clone NDA8694-3 (-3). Potato seed sources and procedures for planting, fertilization, irrigation, and control of insects and weeds were similar to those in field study 1. Methods involving assays of the pathogen from plant tissue, inoculum densities in soil, disease severity ratings, and yield data were also obtained in the same manner for both field studies.

Assays for *V. dahliae* in plant tissue. When symptoms of Verticillium wilt became evident during the last week of August each year, 30 stems were collected at intervals of approximately 60 cm from the center two rows in each plot. These samples were collected from the terminal 7.5 cm of each stem. The stems were selected without regard to appearance and included stems with and without wilt symptoms. Stem segments were stored overnight at 4 C, surface-disinfested in 0.5% NaOCl for 10 sec, and air-dried in clean paper bags at 18–21 C for 21–35 days. Stems from each plot were bulked, ground with a Wiley mill, and passed through a 40-mesh screen. Stem tissue was assayed for *V. dahliae* with the Anderson Sampler and a selective medium (NPX) (6). A total of five 10-mg tissue subsamples was distributed to five plates of NPX medium for each respective sample. After incubation for 21 days at 18–21 C, *V. dahliae* colonies were counted at 15–90×. Identification of typical *V. dahliae* colonies on NPX agar was further confirmed after transfer to potato-dextrose agar (PDA) and incubation for 3 wk.

Stem bases and roots were assayed for *V. dahliae* on 8 August 1980. Thirty representative stem bases per plot were collected, and a cross section from each (1 mm thick) was removed from an area ~1.3 cm above the soil surface. Samples were disinfested, air-dried, ground, and assayed on a selective medium as described above. Sixty roots per plot were collected from a region 2.5 cm from the base of the below-ground stem. All roots 1–2 mm in diameter were disinfested with 0.5% NaOCl for 10 sec. Tissue from these roots was collected 2.5–5.0 cm from the root bases, bulked, and plated with the Anderson Sampler onto NPX agar. From the bulked sample for each plot, a total of five 10-mg subsamples was assayed onto five plates of NPX.

Assays of soil for *V. dahliae*. During May–June of 1978–1988, 18 soil samples of 2-cm core size were collected from the top 23 cm of soil profile of potato hills within the center 1.8 × 7.6 m region of each plot for field studies 1 and 2. At time of collection, subsamples from each plot were bulked and separately mixed in a clean 8-L container. These samples were air-dried for 6 wk at 20–25 C. Samples from each plot were then separately remixed in 39 × 47 cm plastic bags. From this sample, six 80-g subsamples were randomly collected and again mixed. Samples from this third mixing were then passed through a 250 μm (60-mesh) screen, both to remove organic matter and to standardize the particle size. Final samples were mixed again; and for each plot, five subsamples (50 mg each) from the final mixing were plated, each onto a separate plate of NPX medium, with the Anderson Sampler procedure for soil assay (6). After incubation for 21 days at 18–21 C, plates were washed gently to remove soil and examined at 15–90×. Microsclerotia in *V. dahliae* colonies resembled the size and appearance described by others (13,14).

Disease assessment. Wilt symptoms were evaluated each year at season end (28 August–2 September). To separate Verticillium-like symptoms from other factors that may induce similar symptoms (e.g., drought stress, nutrient deficiency, senescence, etc.) the terminal 7.5 cm of stem tissue was assayed for *V. dahliae* in the manner described previously. With the exception of 1983 and 1984, when symptoms were not severe, plants were evaluated for Verticillium wilt by determining incidence of plants showing severe wilt symptoms (>75% of foliage with symptoms), as described previously (11). To determine disease incidence, thirty stems per plot were collected at ~60-cm intervals within the center two rows of each plot. In 1983 and 1984, symptoms were expressed as the percentage of stems in each plot (50 stems evaluated per plot) showing the presence of wilt symptoms on the uppermost 15 cm of the stem.

Incidence of vascular discoloration was determined from representative samples of 30 stems per plot on 15 August 1979 and 21 August 1980. A cross section of each stem was taken 2.5 cm above the soil line. Sections were examined visually for vascular discoloration, and the number with two or more discolored vascular bundles was counted from each 30-stem sample.

Harvesting and grading. With the exception of an early sampling on 11 August 1988 in field study 2 from 1.5 m of row, potatoes were harvested each year between 25 September and 7 October from a total of 10.7–15.2 m (depending on year) of the center rows to avoid border effects in each plot. Leaves and stems were harvested with the early sampling to provide additional measurements of plant growth. Tubers were washed and graded according to standard methods (1).

Data analyses. Data involving stem assays of *V. dahliae* were significantly and positively skewed. Therefore, the analysis of variance of *V. dahliae* per gram of tissue was performed on transformed $\log_{10}(n + 1)$ values. Data on percent infection were analyzed after arcsine-square root transformation. In all cases, means were calculated from the transformed data and then reconverted to original units. The effect of treatments on changes in cfu per gram of soil over time was assessed according to a Latin square repeated-measures analysis. To adjust for a nonrandom assignment of years, since years occurred in sequence, the Greenhouse-Geisser adjustment was used (17) to ensure that all comparisons for varieties among years were valid. All analyses of variance were conducted in accordance with the experimental design.

RESULTS

Verticillium wilt and *V. dahliae* inoculum during continuous cropping. *Incidence of wilt.* When the potato clones and cultivars in field study 1 were grown for five consecutive seasons (1977–1981), the incidence of Verticillium wilt was consistently lower for the more resistant clones (Targhee, -51, and -4) than for either Russet Burbank or Butte (Table 1). During each of the 5 yr, the wilt incidence for both -51 and -4 was negligible (1% or less). The wilt incidence for Targhee ranged from 5 to 13% with continuous cropping, while the incidence of wilt for Russet Burbank or Butte consistently exceeded 40% by late August and approached or exceeded 60% in 1980 and 1981. Similarly, in field study 2 (1983–1987), wilt incidence with the resistant clone (-51) was significantly less than with Russet Burbank in all years but the first (Table 2). In both studies, the incidence of late-season wilt in the resistant clones ranged from 0 to 7% (average over years, 1.2%) compared with Russet Burbank, for which incidence ranged from 11 to 69% (average over years, 50.3%).

Propagules of V. dahliae *in apical stems.* In field study 1 (Table 1), actual numbers of cfu in apical stem tissue varied from year to year, but the relative differences among clones remained the same. With the exception of 1978, significantly fewer *V. dahliae* cfu were recovered from apical stem tissue of the highly resistant clones (-51 and -4) than from Russet Burbank or Targhee. During all years, fewer cfu were recovered from the -51 and -4 clones than from Butte. Results of field study 2 also showed fewer *V. dahliae* cfu in the -51 clone than in Russet Burbank (Table 2). With continuous cropping from 1983 to 1987, the

TABLE 1. Response of potato clones to Verticillium wilt when grown for five consecutive seasons (field study 1, 1977–1981)

Potato cultivar or clone	1977	1978	1979	1980	1981
Wilt incidence[v] (% stems)					
Russet Burbank	55.0 a[w,x]	47.0 a[w]	45.0 a	69.0 a	59.0 a
Butte	51.0 a	41.0 a	39.0 a	60.0 a	58.0 a
Targhee	13.0 b	11.0 b	1.8 b	11.0 b	4.6 b
A66107-51	0.0 c	0.1 c	0.1 b	0.3 c	0.0 c
A68113-4	0.1 c	0.2 c	0.0 b	0.1 c	0.1 bc
V. dahliae in apical stems (cfu/g tissue)[y]					
Russet Burbank	94 b[w]	290 ab[w]	7,650 b	1,000 a	2,790 a
Butte	600 a	1,750 a	26,000 a	4,600 a	6,560 a
Targhee	22 b	52 abc	3,810 b	460 a	570 b
A66107-51	0 c	8 bc	550 c	8 b	1 c
A68113-4	0 c	3 c	150 d	3 b	1 c
	Correlations of % wilt incidence with *V. dahliae* cfu in stems[v] (r values)[z]				
cfu/g	0.539*	0.661**	0.620**	0.717***	0.881***
$\log_{10}$cfu/g	0.845***	0.726***	0.649**	0.737***	0.784***

[v] Percent stems dead or dying and exhibiting Verticillium wilt symptoms. Stems collected during the last 10 days of August each year.

[w] Data previously published (11,12).

[x] Different letters denote significant differences within a column, $P = 0.05$. Analyses of variance and means based upon arcsine square root transformations for wilt data and $\log_{10}$(cfu + 1) for inoculum density data.

[y] Colony forming units (cfu) quantified from uppermost 7.5 cm of stems. Lowest limit of detection was 20 cfu/plot, with exception of 1981 when limit of detection was enhanced to 10 cfu/plot.

[z] *, **, and *** denote significant relationships at $P = 0.05$, 0.01, and 0.001, respectively. Twenty comparisons.

TABLE 2. Response of potato clones to Verticillium wilt when grown for five consecutive seasons (field study 2, 1983–1987)

Potato cultivar or clone	1983	1984	1985	1986	1987
Wilt incidence[w] (% stems)					
Russet Burbank	11[x]	38 a[y]	69 A	52 A	58 A
A66107-51	7	0 b	7 B	0 B	0 B
V. dahliae in apical stems (cfu/g tissue)					
Russet Burbank	360 a	120 a	525 a	6,060 A	3,115 A
A66107-51	35 b	0 b	20 b	15 B	0 B
	Correlations of % wilt incidence with *V. dahliae* cfu in stems (r values)[z]				
cfu/g	0.554 ns	0.608 ns	0.788*	0.970***	0.946***
$\log_{10}$cfu/g	0.394 ns	0.825*	0.839**	0.908**	0.974***

[w] Percent stems dead or dying and exhibiting Verticillium wilt symptoms. Stems collected during the last 10 days of August each year.

[x] Differences between Russet Burbank and A66107-51 not significant.

[y] Different letters denote significant differences within a column, $P = 0.05$ for lowercase letters a and b, and $P = 0.01$ for uppercase letters A and B. Analyses of variance and means based upon arcsine square root transformations for wilt data and $\log_{10}$(cfu + 1) for inoculum density data.

[z] ns = Not significant. *, **, and *** denote significant relationships at $P = 0.05$, 0.01, and 0.001, respectively. Eight comparisons.

number of cfu in -51 stems was significantly lower than that in Russet Burbank.

Correlations between wilt incidence and V. dahliae *propagules.* Numbers of cfu per gram in potato stems correlated significantly ($P < 0.05$ or 0.001 depending on the year) with wilt incidence except during 1983, when both wilt incidence and *V. dahliae* colonization in potato stem tissue were low (Tables 1 and 2). Correlations of percent vascular discoloration with wilt percentages were highly significant ($P = 0.001$) during each year.

The relationship of wilt resistance to numbers of *V. dahliae* propagules in potato stems was most evident with assays from the terminal stem tissue. Correlations of vascular discoloration and propagules from apical stem tissue were greater than from lower stem tissue (apical stem sections, $r = 0.717$ [$P = 0.001$]; basal stem sections, $r = 0.498$ [$P = 0.05$]; below-ground stems, $r = 0.309$, not significant). These observations indicated that assays from stem apices are most reliable for the quantification of *V. dahliae* infection.

TABLE 3. Potato yields during five years of continuous cropping[y]

	Yield (metric tons/ha)				
Field study 1	1977	1978	1979	1980	1981
Total yield					
Russet Burbank	28 cd[z]	19 b	25 cb	13 cd	24 bc
Butte	31 bc	24 b	23 c	12 d	25 bc
Targhee	25 d	20 b	22 c	16 c	20 c
A66107-51	35 ab	24 b	29 b	27 b	30 b
A68113-4	38 a	35 a	37 a	32 a	37 a
U.S. no. 1 yield					
Russet Burbank	17 b	10 a	18 b	5 d	12 b
Butte	21 ab	13 a	17 b	3 d	14 b
Targhee	16 b	13 a	17 b	9 c	13 b
A66107-51	24 a	16 a	20 ab	16 b	13 b
A68113-4	26 a	20 a	27 a	21 a	22 a
Field study 2	1983	1984	1985	1986	1987
Total yield					
Russet Burbank	39 a	25 a	27 a	20 a	22 a
A66107-51	38 a	33 b	36 b	27 a	34 b
U.S. no. 1 yield					
Russet Burbank	24 a	12 a	14 a	6 a	9 a
A66107-51	19 b	20 b	21 b	13 b	22 b

[y] Yields expressed on a per hectare basis represent a transformation of yield data originally collected from plants in two rows (10.7–15.2 m long) per plot.

[z] Different letters within columns denote significant differences at $P = 0.05$ level by Duncan's multiple range test. Each value shows the mean of four observations.

Yield. The potato clones with the highest degree of *V. dahliae* resistance (-51 and -4) in field study 1 had the highest yields and acceptable grade quality (Table 3). Total yields of -51 ranged from 20 to 110% higher than yields of Russet Burbank during the 5-yr period of study 1; while U.S. no. 1 yields for -51 ranged from 4 to 220% higher than for Russet Burbank during the same period. Likewise, total yields of -4 ranged from 38 to 140% higher than yields of Russet Burbank, and U.S. no. 1 yields were 55 to 310% higher. Over the entire 5-yr period of continuous cropping, however, only the -4 clone consistently produced yields that were significantly greater than those of Russet Burbank. Similarly, in study 2, as wilt severities increased over time with continuous cropping (1983–1987), the resistant -51 clone continued to produce greater yields than Russet Burbank each year from 1984 to 1987 by 30–54% for total yield and 51–138% for U.S. no. 1 yield.

Changes in soilborne inoculum densities with continuous cropping. Tables 4 and 5 summarize the repeated measures analyses of variance for studies 1 and 2, respectively. The time × treatment interaction terms were partitioned into polynomial components, which indicated that the slopes differed among potato clones over time. These ANOVA contrasts reflect the trends shown by Figures 1 and 2, respectively.

In study 1, both time and time × treatment interactions were significant. Figure 1 shows that a consistent decrease of inoculum densities occurred throughout all treatments during years 2 and 3; while during the last 2 yr, inoculum densities increased in plots in which Russet Burbank, Butte, and Targhee were grown. This is reflected by a significant linear time × treatment interaction for both cfu per gram of soil and log (cfu/g soil + 1). Mean inoculum densities differed among years. In both 1979 and 1980, inoculum densities were lower ($P = 0.01$) than in 1978. From 1980 to 1981, the mean inoculum density increased significantly from 47 to 122 ($P = 0.01$); and between 1981 and 1982, it increased again from 122 to 182 ($P = 0.01$).

Depending on the potato clone, inoculum density varied from year to year. While inoculum density increased in plots with the susceptible Russet Burbank, Butte, or Targhee from 1978 to 1982 ($P = 0.01$), no increase occurred with the resistant -51 and -4 clones. Soilborne inoculum of *V. dahliae* increased between 1981 and 1982 with Russet Burbank and Targhee ($P = 0.01$), but no increase occurred with the other clones (Butte, -51, and -4). In 1981, plots in which Russet Burbank had been grown had significantly greater ($P = 0.01$) inoculum densities than did all other plots (-51, -4, Butte, and Targhee); and plots with Butte and Targhee had greater inoculum densities than did those cropped with either -51 or -4. At the conclusion of field study 1, the plots cropped continuously with the most resistant clones had the lowest inoculum densities.

TABLE 4. Repeated measures analyses of variance (field study 1)

		cfu/g Soil		Log (cfu/g soil)	
	df	Mean square	$P > F$	Mean square	$P > F$
Source					
Treatment	4	38,809	0.0001	3.667	0.0001
Reps	3	4,774	0.1513	0.356	0.2863
Error (a)	12	2,255		0.252	
Time	4	61,436	0.0001	8.725	0.0001
Time × treatment	16	5,627	0.0025	0.253	0.0394
Time × rep	12	1,008	0.6976	0.077	0.7019
Error (b)	48	1,456		0.113	
Total	99				
Time					
Linear	1	93,918	0.0001	8.915	0.0001
Quadratic	1	140,045	0.0001	13.719	0.0001
Cubic	1	6,763	0.0711	7.842	0.0001
Lack of fit	1	5,020	0.0130	4.426	0.0002
Time × treatment					
Time linear × treatment	4	15,477	0.0032	0.657	0.0204
Time quadratic × treatment	4	4,397	0.0537	0.068	0.5396
Time cubic × treatment	4	796	0.7630	0.217	0.0440
Lack of fit	4	1,836	0.0569	0.070	0.7707

TABLE 5. Repeated measures analyses of variance (field study 2)

	df	cfu/g Soil		Log (cfu/g soil)	
		Mean square	$P > F$	Mean square	$P > F$
Source					
Row	3	76.9	0.2471	1.659	0.3502
Column	3	106.6	0.1569	2.147	0.2620
Treatment	3	1,090.8	0.0008	6.171	0.0464
Error (a)	6	42.7		1.250	
Time	5	638.6	0.0002	3.563	0.0029
Time × row	15	134.7	0.0419	0.827	0.4260
Time × column	15	97.1	0.1174	1.004	0.3198
Time × treatment	15	346.7	0.0005	1.638	0.1154
Error (b)	30	48.7		0.766	
Total	95				
Time					
Linear	1	1,735.0	0.0001	2.401	0.0943
Quadratic	1	839.2	0.0120	5.217	0.0477
Cubic	1	462.4	0.0526	1.592	0.0245
Quartic	1	47.6	0.3467	3.228	0.2137
Lack of fit	1	108.7	0.1678	5.377	0.0187
Time × treatment					
Time linear × treatment	3	1,109.0	0.0001	3.117	0.0431
Time quadratic × treatment	3	479.3	0.0205	2.652	0.1088
Time cubic × treatment	3	80.4	0.4514	1.096	0.0295
Time quartic × treatment	3	14.3	0.8154	0.067	0.9882
Lack of fit	3	50.4	0.4058	0.397	0.5590

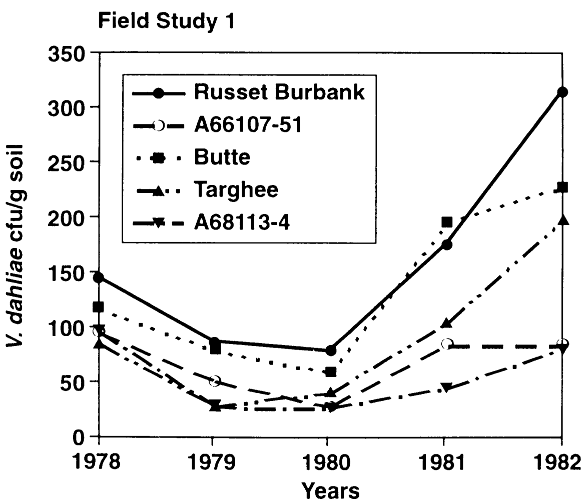

Fig. 1. Inoculum density of *Verticillium dahliae* in plots planted with the same potato clones over 5 yr. Inoculum density was quantified in May of each year.

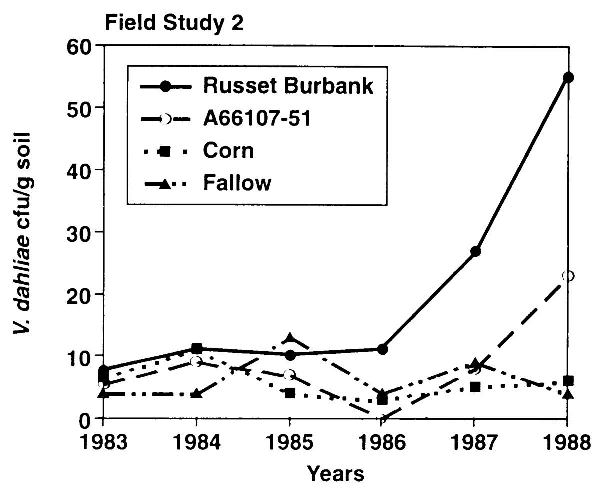

Fig. 2. Inoculum density of *Verticillium dahliae* in fallow plots or plots planted with a susceptible potato cultivar (Russet Burbank), a resistant clone (A66107-51), or corn for 5 yr. Inoculum density was quantified in June of each year.

In study 2, inoculum density in corn and fallow plots remained low and stable during 1983–1988. However, the continuous cropping of Russet Burbank appeared to result in a buildup of *V. dahliae* inoculum in 1987 and 1988, whereas the resistant -51 clone resulted in no increase until 1988 (Figure 2). A significant time linear × treatment term for both the actual and log values occurred (Table 5). A significant time quadratic × treatment term occurred for the actual values, whereas a significant time cubic × treatment interaction occurred for the log values.

As in study 1, continuous cropping of Russet Burbank in study 2 produced a more rapid increase of *V. dahliae* cfu in soil than did the highly resistant potato clone (-51). Mean inoculum densities differed among years. In 1987, inoculum density was significantly greater ($P = 0.05$) than in 1983 (increase from 6 to 12 cfu/g); and from 1987 to 1988, a further increase occurred (from 12 to 22 cfu/g, $P < 0.01$).

Year-to-year variation in inoculum density was clone dependent. Inoculum density increased more rapidly in plots planted with Russet Burbank than in plots with the more resistant clone -51. During 1987, *V. dahliae* populations in Russet Burbank plots increased from 8 cfu/g of soil to 27 cfu/g ($P = 0.01$), while no increase occurred in -51, corn, or fallow plots (Fig. 2). Between 1987 and 1988, inoculum density in plots with Russet Burbank increased again from 27 to 55 cfu/g, while in the -51 plots there was a significant increase ($P = 0.01$) from 8 cfu/g in 1987 to 23 cfu/g in 1988. During the period from 1983 to 1988, inoculum densities in corn or fallow plots did not change. During 1983–1985, *V. dahliae* populations did not differ among plots; whereas in 1986–1988, the inoculum density in -51 plots was significantly less than in Russet Burbank plots.

Field bioassay with Russet Burbank. *Incidence of severe wilt.* When Russet Burbank was planted in all plots of study 1 during 1982, the incidence of severe wilt was significantly less in plots where -51 and -4 had been grown during the previous 5 yr compared to plots continuously cropped with Russet Burbank or Butte (Table 6). The wilt incidence was also less ($P = 0.05$) where -51 had been grown than where Targhee had been grown.

Similar results were also observed with study 2 (Table 6). When Russet Burbank was planted in all plots in 1988, wilt incidence in plots with a history of -51 (45%) was approximately half that

TABLE 6. Verticillium wilt variables and yield of a susceptible (Russet Burbank) or highly susceptible (NDA8694-3) potato clone in plot following 5 yr of continuous cropping or fallow

Year, potato cultivar or clone	Cropping history preceding 5 yr	*V. dahliae* (cfu/g soil)	Severe wilt incidence[s] (% plants)	*V. dahliae* (cfu/g tissue)	Yield (metric tons/ha)[t]	
					Total	U.S. no. 1
Study 1						
1982–Russet Burbank	Russet Burbank	315 a[u]	34 ab[v,w]	2,400 a[x]	21 ab	6 ab
	Butte	228 b	43 a	2,700 a	18 b	4 b
	Targhee	199 b	23 bc	1,700 ab	22 ab	8 a
	A66107-51	85 c	8 d	680 c	22 ab	7 ab
	A68113-4	81 c	18 cd	1,050 bc	25 a	7 ab
Study 2						
1988–Russet Burbank	Russet Burbank	55 a	80 a[w,y]	3,495 a[x]	25 a	9 a
	A66107-51	23 b	45 b	2,665 a	28 a	9 a
	Corn	6 c	13 c	340 b	43 b	20 b
	Fallow	4 c	32 b	685 ab	44 b	23 b
1988–NDA8694-3	Russet Burbank	55 a	80 a[w,z]	8,515 a[x]	21 a	8 a
	A66107-51	23 b	39 b	3,485 b	24 a	9 a
	Corn	6 c	7 c	1,125 c	38 b	15 b
	Fallow	4 c	6 c	815 c	37 b	16 b

[s] Values represent mean percent plants exhibiting >75% foliage with wilt symptoms (50 stems evaluated/plot).
[t] Yields expressed on a per hectare basis represent a transformation of yield data originally collected from plants in two rows (15.2 m long) per plot.
[u] Different letters denote significant differences within a column, $P = 0.05$.
[v] Wilt data taken 20 August 1982.
[w] ANOVA based on arcsine square root transformations for wilt data.
[x] ANOVA based on $\log_{10}(\text{cfu} + 1)$ transformations of inoculum density in stems.
[y] Wilt data taken 17 August 1988.
[z] Wilt data taken 8 August 1988.

TABLE 7. Correlations between wilt incidence and yield of bioassay potato crops grown in plots with different 5-yr cropping histories

Yield variable	*r* Values[w]	No. of comparisons
Study 1–1982		
Russet Burbank[x]		
Total tuber yield	−0.472*	20
U.S. no. 1 tubers	−0.614**	20
Undersized tubers	0.689**	20
Study 2–1988		
Russet Burbank[y]		
Total tuber yield	−0.812***	16
U.S. no. 1 tubers	−0.785***	16
Undersized tubers	0.527*	16
NDA8694-3[z]		
Total tuber yield	−0.896***	16
U.S. no. 1 tubers	−0.873***	16
Undersized tubers	0.814***	16

[w] * = Significant relationship at $P = 0.05$; **, at $P = 0.01$; ***, at $P = 0.001$.
[x] Correlated with wilt incidence on 20 August 1982.
[y] Correlated with wilt incidence on 11 August 1988.
[z] Correlated with wilt incidence on 8 August 1988.

in plots that had been planted with Russet Burbank for 5 yr (80%). Disease incidence did not differ significantly between plots with a history of either -51 or the fallow treatment. The lowest disease incidence occurred in plots with a history of continuously cropped corn. This relationship was further tested by growing NDA8694-3 (-3), a potato clone with a greater susceptibility to Verticillium wilt than Russet Burbank (18). Even with great susceptibility, the results remained similar. When plots were continuously planted with -51, disease incidence was 44% less than in plots that had been planted with Russet Burbank. The lowest incidence of disease was in plots with a 5-yr history of corn or left fallow.

Reproduction of V. dahliae *in plants.* Wilt incidence in the potato crops that were grown as a bioassay in all plots was highly correlated with the occurrence of *V. dahliae* propagules in apical stems. Linear correlation coefficients between percent wilt incidence and log (cfu + 1) per gram of tissue in apical stems were, in 1982 (Russet Burbank), $r = 0.823$ ($P = 0.001$); in 1988 (Russet Burbank), $r = 0.680$ ($P = 0.01$); and in 1988 (-3), $r = 0.941$ ($P = 0.001$). During 1982, when Russet Burbank was planted in all plots, stems of plants in plots with a history of either highly resistant potato clone (-51 and -4) had significantly fewer propagules than stems collected from plots with a history of either Russet Burbank or Butte. Numbers of propagules in Russet Burbank stems also were less in the former -51 plots than in the former Targhee plots.

Data from study 2 in 1988 with the highly susceptible -3 clone corroborated the data from study 1. Following -51 for 5 yr, numbers of *V. dahliae* propagules per gram of -3 stem tissue were significantly less than when -3 followed 5 yr of Russet Burbank (Table 6). Following either corn or fallow plots, numbers of *V. dahliae* propagules per gram of -3 stem tissue were less than following 5 yr of either Russet Burbank or -51 plots. Although trends were similar to the -3 stem tissue, the numbers of *V. dahliae* propagules in Russet Burbank stems did not differ between plots where either -51 or Russet Burbank had grown for 5 yr. Following 5 yr of corn, however, the numbers of *V. dahliae* propagules in Russet Burbank apical stem tissue were significantly less than following 5 yr of -51 or Russet Burbank.

Yield. Russet Burbank yield in 1982 was 39% higher ($P = 0.01$) following 5 yr of the highly resistant -4 clone than following the highly susceptible Butte cultivar (Table 6). U.S. no. 1 yield for Russet Burbank following the more resistant Targhee cultivar was twice that following Butte. In study 2, continuous cropping of either Russet Burbank or -51 was not followed by yield differences between the Russet Burbank and -51 plots in 1988, when either Russet Burbank or -3 were grown in all plots. Yields of either Russet Burbank or -3 in either the corn or fallow plots, however, were significantly greater by 54–76% when compared with either of the 5-yr potato plots. Increases of U.S. no. 1 yields ranged from 78 to 156% in corn and fallow plots when compared with plots with 5 yr of potato.

Wilt incidence was correlated with both total yield and U.S. no. 1 yield in both the 1982 and 1988 bioassays (Table 7).

Russet Burbank plants grown in plots in which -51 had been grown for 5 yr had greater total dry weights than those grown in former Russet Burbank plots (Table 8). Although early tuber yields for Russet Burbank did not differ significantly between the former -51 and Russet Burbank plots, root and vine dry weights of Russet Burbank were significantly greater in former -51 plots

TABLE 8. Plant dry weights and number of stems and tubers of Russet Burbank plants following 5 yr of cropping with potato or corn or held fallow (field study - 11 August 1988)

Cropping history preceding 5 yr 1983–1987	Means/1.5 m of row					
	Number		Dry weight (g)			
	Stems	Tubers	Tubers	Roots	Tops	Total
Russet Burbank	17.3[y]	38.3[y]	626 b[z]	55 bc	406 b	1,086 c
A66107-51	16.8	38.3	773 b	64 a	540 a	1,376 b
Corn	18.3	35.5	1,032 a	61 ab	582 a	1,675 a
Fallow	15.0	36.0	1,031 a	51 c	396 b	1,478 b

[y] Differences not significant.
[z] Different letters denote significant differences within a column at $P = 0.05$ via Duncan's multiple range test.

than in former Russet Burbank plots. Stem and tuber numbers did not differ with plot history, but plots in which Russet Burbank had previously been grown had significantly lower total dry weight of tubers, roots, and tops in August than did the former -51, fallow, or corn plots. In plots that had been previously cropped with -51, the root and top dry weights of Russet Burbank were significantly higher than in plots that had been either previously cropped with Russet Burbank or allowed to remain fallow.

DISCUSSION

These experiments provided no evidence for the loss of Verticillium resistance due to the natural selection of more aggressive *V. dahliae* strains. Resistance did not diminish, even though our testing conditions were extreme. Under normal cropping practices, a period of 10–40 yr would be required to complete a cycle involving five potato crops. Typically, one or more nonhost rotation crops would have been grown between each potato crop, and less intensive potato cropping might have further reduced the probability for the selection of highly virulent strains.

Resistance was maintained following several seasons of cropping, and the cropping of Verticillium-resistant potato cultivars prevented the increase of *V. dahliae* inoculum in soil. This in turn appeared to benefit succeeding crops of susceptible cultivars. This relationship between Verticillium resistance and reduced soilborne inoculum had not been demonstrated previously with potato.

At the onset of these studies, we speculated that the incorporation of stems of resistant clones into soil would suppress inoculum production. Although clones -51 and -4 are highly resistant to *V. dahliae,* they are not immune, and their roots and stems were infected. Furthermore, -51 produced 1 t/ha more above-ground stem material than did Russet Burbank. With such an increase in stem mass, the potential existed for an increase rather than a decrease of soilborne inoculum. However, this investigation showed that the buildup of *V. dahliae* was closely related to its degree of reproduction in stem apices, and there was no evidence that *V. dahliae* in roots invaded stem tissue of resistant clones following vine death.

From the outset of both studies, *V. dahliae* inoculum was present throughout all plots, and any movements that may have occurred either with tillage equipment or in irrigation water were regarded as insignificant to the existing populations. There was no indication of a significant movement of *V. dahliae* from plot to plot. The sharp differences of wilt incidence between plots remained the same at the conclusion of both studies (1982 and 1987). Furthermore, the lack of increase of inoculum density in some plots from the beginning to the end of the experiment, and the ability to detect significant changes in inoculum densities in these plots by ANOVA, indicate that little or no significant movement of soilborne inoculum occurred.

It is possible that inconsistent yield benefits with Verticillium suppression could have been due to the interaction of other disease organisms. The presence of *Colletotrichum coccodes* (Wallr.) S.J. Hughes provided one such possibility. The medium and procedure that we used for the assay of *V. dahliae* can also be used for the identification and recovery of *C. coccodes* (11). Since we did not detect this pathogen in apical stems during our studies, we believe the likelihood of a significant presence of *C. coccodes* in this tissue was not high. However, during isolated instances, we did recover it from both below-ground stems and soil (*unpublished*). Although we have no knowledge of the effect of continuous potato cropping on soilborne *C. coccodes* inoculum, the increase of this pathogen might be anticipated. Soilborne *C. coccodes* could have affected yield both directly and by interacting with *V. dahliae* (19). The only pathogenic nematode identified was *Pratylenchus neglectus,* which we have not shown to interact significantly with *V. dahliae* or to reduce tuber yield (10).

Factors other than diseases and pests might also influence yield after 5 yr of continuous cropping (22). In a long-term cropping sequence of 10 or 20 yr, which was not possible to duplicate in this study, a resistant potato would be alternated with a nonhost crop. Although alternate cropping in a long-term sequence of resistant potato clones with nonhost crop rotations has not been tested, our results provide indirect evidence that this combination might significantly lower inoculum density. Not only may losses due to Verticillium wilt be limited during the years of cropping potatoes, but the use of Verticillium-resistant clones appears to have major possibilities in helping to manage Verticillium wilt in subsequent crops.

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