A Sensitive Method for Quantifying *Verticillium dahliae* Colonization in Plant Tissue and Evaluating Resistance Among Potato Genotypes

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ABSTRACT

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A technique involving a selective nutrient pectate medium and assay of air-dried potato stems with an Andersen sampler provided a means of quantifying *Verticillium dahliae* in plant tissues. From both naturally and artificially inoculated potato cultivars, the recovery of *V. dahliae* from air-dried potato tissue was found to be highly correlated with foliar wilt symptoms. A straight-line relationship for *V. dahliae* recovery with time was demonstrated by this method, and isolations from fresh stem tissue were highly correlated with assays from air-dried tissue. Inoculum levels in soil were similarly correlated with the rate of *V. dahliae* colonization in potato-stem tissue. Greenhouse and field studies with this procedure have consistently indicated two potato clones (A66107-51 and A68113-4) to be much more resistant to *V. dahliae* than the Russet Burbank cultivar; plants with resistance showed an increase in yield. In contrast, Nampa and Butte cultivars were found to be significantly more susceptible.

Additional key words: soilborne pathogens, *Solanum tuberosum*.

Verticillium wilt of potato (*Solanum tuberosum* L.) may be caused by either *Verticillium albo-atrum* Reinke & Berth. or *V. dahliae* Kleb. In Idaho and other arid growing regions of the West, this disease is caused by *V. dahliae* (2). For purposes of diagnosis, the disease symptoms on potato are not reliable. Isaac and Harrison (7) state that "the only true characteristic symptom, that of unilateral chlorosis and necrosis, is morphologically indistinguishable from senescence symptoms, and is not produced until maturity of the host (the potato), and then often a week or so before normal senescence." Thus, the possibilities for incorrect diagnosis are many. This being the case, there is a vital need to determine the degree of *V. dahliae* colonization in potato tissue if effects of treatments (eg, pesticide, cultural practices, cultivars) are to be accurately evaluated.

Currently used methods involving manual isolations from freshly collected stem tissue, dilution plating, or the counting of microsclerotia in stems (9) are laborious and require immediate evaluation. Because time frequently is limited during the growing season, it would be a valuable advantage if samples could be collected and stored until more time is available for critical evaluation. Easton (G. D. Easton, *personal communication*) indicated that *V. dahliae* could survive air-drying and be successfully recovered from potato stem tissue by a dilution plating technique. This presented the possibility that stem samples could be collected in the summer, air-dried, and assayed during the fall and winter. Our preliminary observations showed *V. dahliae* counts from air-dried stem tissue (obtained by dilution plating) to be closely correlated with conventional, fresh-tissue isolations.

Harrison and Livingston (5) described a method using an Andersen sampler for *V. dahliae* assay from air-dried soil, and this procedure was successfully utilized in studies on the epidemiology of soilborne *V. dahliae* in Colorado (8). Because the method involved fewer steps and had apparent advantages for more reliable quantification, it was believed that the method of Harrison and Livingston might be adapted to an assay of air-dried potato tissue.

Butterfield and DeVay (1) modified this method to include a selective medium, nutrient pectate agar (NPX), for *V. dahliae* identification. Personal experience with this medium indicated many advantages, including good colony separation, development of microsclerotia, and species separation. Among Idaho potato fields, *V. tricorpus* Isaac (avirulent to mildly virulent on potato) is often associated with potato-stem tissue. On certain commonly used media (eg, potato-dextrose agar [PDA] and water agar), the microsclerotia of *V. tricorpus* and *V. dahliae* are similar in size, and the appearance of *V. tricorpus* colonies may be easily confused with *V. dahliae*. In contrast, on the NPX medium described by Butterfield and DeVay the colonies of *V. tricorpus* are readily distinguishable from *V. dahliae* (3).

The first objective of our study was to determine whether these techniques (1,5) could be used to assay *V. dahliae* in air-dried potato stems. The second objective was to relate *V. dahliae* colonization, determined by these techniques, to the severity of symptoms for Verticillium wilt, yield, and grade of potatoes. This report describes the application of these objectives to a wide range of potato genotypes with concomitant evaluations for resistance. An abstract describing a portion of this work has been published (4).

MATERIALS AND METHODS

Assays of *V. dahliae* colonization in potato stem tissue among field plots. *1975 Survey study.* Twenty potato genotypes were grown in a Declo loam soil and compared among 80 plots for *V. dahliae* field resistance at the University of Idaho Research and Extension Center, Aberdeen, ID. Plots consisted of a single row (5.1 m long) with a 0.9 m spacing between rows. Potatoes were mechanically planted with an assist feed planter on 15 May with a spacing between hills that approximated 25 cm. Irrigation was by sprinkler, and fertilizer and insecticide were applied in accordance with University of Idaho recommendations.

On 18 August, stems were randomly collected from each plot (10 stems per plot). At the time of collection all stems appeared green and succulent with no external evidence of fungal development or macroscopic evidence of soil. Stem sections were cut from a region located 5–10 cm from the ground line. Samples were washed with distilled tap water, air-dried in clean paper bags at 18–21° C for 10 wk, ground with a Wiley mill, and passed through a 40-mesh screen. Between the grinding of each sample, the mill was thoroughly cleaned using a vacuum cleaner to remove all debris

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TABLE 1. Field susceptibility of potato cultivars to *Verticillium dahliae* and relationships to yield in 1976

Cultivars	Isolations of *Verticillium dahliae* from stem apices: Fresh tissue % recovery[s] 26 Aug	From stem apices: Air-dried tissue[t,u] 26 Aug	From stem base: Air-dried tissue[v] 17 Aug	Percent plants with foliar wilt symptoms[w] 28 Aug	Yield and grade: Q/ha[x]	Yield and grade: % U.S. No. 1 tubers
Nampa	59.3 a[y,z]	3.6724 a[y,z]	2.6950 a[y]	65 a[y,z]	258 b[y]	47 a[y]
Butte	26.0 b	2.9489 b	2.6009 ab	49 b	268 b	53 ab
R. Burbank	16.7 c	1.8862 c	2.1205 ab	47 b	264 b	58 b
Targhee	4.3 d	1.7325 c	1.8862 b	8 c	221 a	67 c
A66107-51	0.3 e	0.4407 d	0.5675 c	1 d	327 c	76 d
A68113-4	0.0 e	0.0000 d	0.7095 c	3 cd	349 c	75 d

[s] Each value represents mean percentage value of 300 isolations (one isolation per stem; 50 stems per plot collected). Isolations were made from upper 1.0 mm of stem apices.
[t] $Log_{10}(prop + 1)/g$.
[u] Assays from stem samples represent mean values from composite samples of 30 stems per plot collected from the uppermost 2.5 cm of potato stems. Samples were air-dried for 7 wk before assay procedure.
[v] Assays from stem samples represent mean values from the base of stems within a region approximating 7.5–10.0 cm from soil line from composite samples of 12 stems per plot collected 17 August.
[w] Fifty stems per plot were randomly selected, and each stem was evaluated for symptoms typical of *Verticillium* wilt within the upper 15 cm.
[x] (q/ha)/1.121 = cwt/A.
[y] Different letters denote significant differences ($P = 0.05$) with Duncan's multiple range test.
[z] Values were transformed to arc sine $(\%)^{1/2}$ values before analysis.

TABLE 2. Correlations of *Verticillium dahliae* recovery from freshly collected stem tissue and air-dried tissue

V. dahliae recovery	*r*-Values[a]: Percent wilt	Yield	Percent U.S. No. 1s	No. of comparisons
Percent *V. dahliae* recovery from freshly collected tissue[b]	0.874**	−0.380*	−0.818**	36
Log_{10} propagules *V. dahliae* From air-dried stems (base)[c]	0.723**	−0.593**	−0.648**	36
From air-dried stems (apices)[c]	0.862**	−0.593**	−0.876**	36

[a] * = Significant relationship at $P = 0.05$; ** = significant relationship at $P = 0.001$.
[b] Tissue-plating technique.
[c] Andersen sampler technique.

from the previous sample. Ten weeks after the field collection, stem tissue was analyzed for the presence of *V. dahliae* using the Andersen sampler and selective medium described by Butterfield and DeVay (1). Each tissue sample (10 mg per plate) was applied to five plates of NPX. Plates were incubated at room temperature (18–21° C) for 3 wk and colony counts determined with the aid of a stereo-dissecting microscope. To verify that the colonies were *V. dahliae*, 25 isolates were randomly selected and subcultured to PDA. Since *V. dahliae* sporulates more readily on PDA than on NPX, this medium provided an additional tool for determination of identity.

The longevity of *Verticillium* propagules in ground stem tissues under laboratory storage conditions was examined. Twenty air-dried stem samples were assayed after 2½ mo from the date of collection, and again after an additional 3½ mo.

Foliar wilt symptoms in each plot were evaluated on 12 September to determine the incidence of Verticillium wilt. Disease incidence was based upon a 1–12 scale of disease severity described by Horsfall and Barratt (6), and the mean propagule count-values of four plots for each of 20 potato genotypes were correlated with the incidence of wilt.

1976 Experiment. To further evaluate the sensitivity of the Andersen sampler assay procedure, a field study was conducted at the University of Idaho Research and Extension Center at Aberdeen. The soil type for the site of investigation was a coarse-textured Declo silt loam with a long history of potato production. The level of the *V. dahliae* inoculum was determined by the procedure described by Butterfield and DeVay (1) and was believed to be adequate for maximum disease severity (52 propagules per gram of air-dried soil). Cultural management practices were followed in accordance with standard practices recommended by the University of Idaho.

Plots with potato genotypes shown in Table 1 were arranged in a Latin square design and were 6.6 × 3.6 m in size with a 0.9-m spacing between rows (four rows per plot).

On 17, 25, and 26 August, stem samples were collected in a random manner by treatment and replicate (Table 1). Stems were surface-disinfested in freshly prepared 0.5% NaOCl for 2 min and rinsed in distilled H_2O. Stem samples were air-dried in clean paper bags at room temperature for 7 wk and assayed on NPX agar as previously described.

On 28 August, foliar wilt symptoms were determined. This was accomplished by randomly selecting 50 stems per plot and evaluating for the presence or absence of typical Verticillium wilt symptoms within the uppermost 15 cm of each stem. Data were expressed as the percentages of wilt severity. On 29 September, potatoes were harvested from 12 m of plot row in each plot and were later evaluated for yield and grade.

To determine the percentage of *V. dahliae* recovery from fresh stem tissue, 50 stems were randomly collected from plots on a replicated basis on 25 and 26 August. Stems were surface disinfested in 0.5% NaOCl, and aseptic isolations were made from apical stem tissue (within a region that approximated the uppermost millimeter of apical tissue). One isolation was made on NPX agar from each of the 50 stems collected per plot. In this manner a total of 1,800 isolation attempts was made.

All isolates thought to be *V. dahliae* on NPX were aseptically transferred to PDA and examined following several weeks at room temperature for confirmation of identity. The colonization of *V. dahliae* in air-dried stem tissue was then correlated with recovery from fresh tissue, wilt severity, yield, and grade (Table 2).

1977 and 1978 Experiment. A field study was conducted at the University of Idaho Research and Extension Center at Aberdeen to further investigate the sensitivity of the Andersen sampler assay procedure. The soil type for the site of investigation was a Declo silt loam with a long history of potato production. With a previously observed occurrence of a high incidence of Verticillium wilt during 1974, the natural presence of a high level of *V. dahliae* inoculum in this field was indicated. Cultural management practices were followed in accordance with standard practices recommended by the University of Idaho.

Plots with potato genotypes shown in Table 3 were planted on 23 May in a randomized block design with four replications and were 7.5 × 7.2 m in size with a 0.9 m spacing between rows (eight rows per plot).

On 22 August 1977, 30 stem samples per plot were randomly collected from the uppermost 7.5 cm of potato stems, disinfested, air-dried for 8 wk, and assayed on NPX agar as previously described.

On 31 August 1977, foliar wilt symptoms were determined. This was accomplished by randomly selecting 50 stems per plot and evaluating for the presence or absence of potato stems with severe Verticillium symptoms (stems with evidence of wilt symptoms that exceeded 75% of the stems and foliage). On 2 October 1977, potatoes were harvested from 12 m of plot row in each plot and were evaluated for yield and grade within a month from harvest.

These plots were replanted with the same respective potato genotypes on 15 May 1978.

As previously described, stem samples were collected from plots on 29 August 1978, and foliar wilt symptoms were evaluated. Stem samples were allowed to air-dry for 11 wk before assaying on NPX agar with the Andersen sampler method. On 5 October 1978, potatoes were harvested from 12 m of plot row in each plot and were evaluated for yield and grade within a month from harvest.

1979 Experiment. The rate of *V. dahliae* colonization in stem tissue and relationship of this colonization to soilborne inoculum was studied among replicated plots that were comparing several nitrogen treatments. These plots (involving three treatments and six replications) were located on a coarse-textured loam soil at Egin Bench, ID, in a field with a long history of Verticillium wilt that exceeded 30 yr. Plots were 30 × 10.8 m in size, and were subirrigated throughout the growing season. All plots were treated with nitrogen at 168 kg/ha in the form of either ammonium sulfate, ammonium nitrate, or urea by treatment.

Soil was assayed for *V. dahliae* by randomly collecting 18 subsamples per plot from the uppermost 15 cm of the soil profile and determining propagules by the method of Butterfield and DeVay (1).

Plots of cultivar Russet Burbank were planted on 25 May, and the first stems were collected on 19 July. Additional samples were thereafter collected at weekly intervals until 17 August. A composite of 30 stems was uniformly collected by treatment (five stems per plot) on each collection data. Collections were made from the uppermost 5.0 cm of stems. To reduce variability, samples were collected from the same respective rows in each plot.

As previously described, stems from each collection date were disinfested, air-dried under similar conditions for 10 wk, ground, and assayed on NPX agar by the Andersen sampler method with five plates per sample.

Verticillium wilt symptoms were not observed until 10 August. On this date and during the following week, samples were collected consisting of 45 stems selected at random from each treatment and replicate. These stems were disinfested with 0.5% NaOCl, air-dried for 3 mo, and assayed as previously described to determine *V. dahliae* colonization in stem tissue. Since composite samples of stem samples collected on 19 July showed no evidence of *V. dahliae* in apical stem tissue, increases in *V. dahliae* colonization were calculated to occur from 19 July. With the determination of *V. dahliae* propagules per gram of stem tissue on 10 and 17 August, slopes were calculated on the basis of time (weeks) vs. $\log_{10}$ of *V. dahliae* propagules plus 1 (n + 1) per gram of stem tissue. In this manner, it was possible to correlate the relative increase of *V. dahliae* colonization with soilborne inoculum.

On 17 August plots were indexed for symptoms of Verticillium wilt on a scale of 1 to 12 as described by Horsfall and Barratt (6). These indices were correlated with levels of inoculum in soil and with *V. dahliae* colonization in potato-stem tissue. Propagule counts from stem tissue were similarly correlated with soilborne inoculum.

Greenhouse evaluation of *Verticillium* resistance. Potato genotypes from among those surveyed were selected for further evaluation (Table 4). These genotypes have been previously observed to tuberize and bulk at similar times from time of planting and are considered to be of similar maturity classes. Potato seed that had been shown by isolations from tuber stem-ends to be free of *V. dahliae* were obtained from the Idaho foundation seed farm at Tetonia, ID. Before planting, the seed was disinfested for 2 min in 0.5% NaOCl, rinsed in tap water, and planted in pathogen-free, U.C. mix, potting soil. Seed was allowed to grow in flats for approximately 6 wk. Sprouts (approximately 10–15 cm in height) were removed, rinsed, and held for a maximum period of 3 hr under wet paper towels until time of inoculation. During the process of sprout removal from mother tubers, care was taken to avoid root injury.

Inoculations were accomplished by dipping roots into 10^7 viable cells per milliliter of *V. dahliae* inoculum, followed by immediate planting. Four 15 × 17-cm pots were utilized for each treatment and

TABLE 3. Field susceptibility of potato cultivars to *Verticillium dahliae* and relationships to yield in 1977 and 1978

Cultivars	Assay of *V. dahliae*[v,w] 1977	Assay of *V. dahliae*[v,w] 1978	Plants with foliar wilt symptoms (%)[x,y] 1977	Plants with foliar wilt symptoms (%)[x,y] 1978	Yields (total q/ha) 1977	Yields (total q/ha) 1978
Butte	2.7781 a[z]	3.2444 a[z]	51.0 ab[z]	41.5 a[z]	309.1 bc[z]	242.5 a[z]
R. Burbank	1.9790 b	2.4680 ab	55.0 a	46.8 a	276.6 ab	192.3 a
Targhee	1.3581 b	1.7214 abc	13.0 bc	10.8 b	246.7 a	204.2 a
A66107-51	0.0000 c	0.9676 bc	0.0 c	0.5 c	354.1 cd	241.9 a
A68113-4	0.0000 c	0.6042 c	0.5 c	0.8 c	382.8 d	348.2 b

[v] $\log_{10}$ (prop + 1)/g of air-dried stems.
[w] Stems were collected from uppermost 7.5 cm on 22 August 1977 and 29 August 1978 and were assayed by Andersen sampler technique after air-drying for 8 and 11 wk, respectively.
[x] Wilt data were determined on 31 August 1977 and 29 August 1978.
[y] Percentage values were transformed to arc sine $(\%)^{1/2}$ before analyses.
[z] Different letters denote significant differences at $P = 0.05$ by Duncan's multiple range test.

TABLE 4. Comparative symptom development, *Verticillium dahliae* colonization, and yield of five cultivars inoculated with *V. dahliae* in the greenhouse

Cultivar	Symptoms: Initial[t]	Symptoms: % Leaves affected[u]	Symptoms: Severe after[v] 36 days	Symptoms: Severe after[v] 44 days	Colonization in air-dried petioles[w,x]	Ratios[y] for mean tuber weight and yield totals: Tuber weight	Ratios[y] for mean tuber weight and yield totals: Total yield
Butte	0.80 a[z]	81.3 a[z]	0.80 a [z]	0.85 a[z]	2.8185 a[z]	0.279 a[z]	0.206 a[z]
R. Burbank	0.40 b	49.5 b	0.30 b	0.95 a	0.0000 b	0.876 c	0.384 a
Targhee	0.05 c	77.6 a	0.70 a	1.00 a	3.1339 a	0.535 ab	0.278 a
A66107-51	0.05 c	21.5 c	0.00 c	0.45 b	0.0000 b	0.986 c	0.660 b
A68113-4	0.05 c	22.1 c	0.10 bc	0.30 b	0.6615 b	0.792 bc	0.579 b

[t] Mean of 20 plants with wilting and/or foliar yellowing among uppermost three leaves 23 days after inoculation.
[u] Percentage of leaves with typical Verticillium wilt 36 days after inoculation.
[v] Mean plants (per pot) with stems with >75% of foliage affected.
[w] Two apical petioles (second petiole from apices) were collected per treatment per replicate (34 days after inoculation) and air-dried for 10 wk before assay.
[x] $\log_{10}$ (prop + 1)/g.
[y] Calculated by dividing the respective yield and tuber weights of inoculated plants by yield and mean tuber weights of noninoculated control plants.
[z] Different letters denote significant differences by Duncan's multiple range test ($P = 0.05$).

replicate, and plants were planted into pathogen-free, U.C. mix soil. Pots were arranged in a randomized block design with five replications.

Inocula used to treat potato sprouts consisted of suspensions of each of two *V. dahliae* isolates in sterile distilled H_2O. These isolates had been recovered from naturally infected plants in Idaho. For each treatment and replicate, two plants were inoculated with each respective isolate. Fresh suspensions of inoculum were used for each replicate. Uninoculated controls (one plant per treatment per replicate) were similarly treated with sterile H_2O.

Plants were grown under greenhouse conditions from February to April, when the normal photoperiod approximated 11–13 hr of daylight.

Symptoms were evaluated at several time periods. At 34 days postinoculation, the second petiole from the apical meristem was uniformly collected from plants of each genotype and replicate inoculated with each isolate. These petioles were dipped for 1–3 sec into freshly prepared 0.5% NaOCl and air-dried at room temperature for 10 wk prior to assay. Petioles from each sample were combined, ground in a vacuum-cleaned Wiley mill, screened through a 40-mesh screen, and 10-mg aliquots were applied to NPX agar with an Andersen sampler. In this manner, five plates per plot were prepared, plates were incubated for 3 wk at 20 C and then evaluated, and data were expressed as the value means.

After 66 days, tubers in pots were harvested, weighed, and the ratios of tuber weights of inoculated plants divided by mean tuber weights on noninoculated plants for each respective genotype were determined.

RESULTS

V. dahliae was recovered from air-dried stem tissue of 20 potato genotypes. In addition, *V. nigrescens* Pethy and *Colletotrichum atramentarium* (Berk. & Br.) Taub. were also recovered. These results demonstrate that the selective medium of Butterfield and DeVay (1) has the potential of providing for the rapid separation of *Verticillium* spp., while simultaneously allowing for the identification and recovery of *C. atramentarium* (syn. *C. coccodes*), the causal organism of the black dot disease of potato.

Comparative determinations of fungal colonization in potato tissue require that tissue be stored under identical conditions for equivalent periods. After 6 mo from time of collection and 3½ mo after sample preparation (grinding of stems), the *V. dahliae* and *C. atramentarium* counts declined by 83 and 76%, but even with these reductions, values were still highly correlated with the original assays ($r = 0.61$, significant at $P = 0.01$).

Recovery of these fungi after 10 wk of air-drying indicated a significant ($r = 0.62$ significant at $P = 0.01$) positive linear correlation for $\log_{10}$ $(n + 1)$ values of *V. dahliae* propagules with wilt severity, whereas no significant relationship with wilt was evident for either *V. nigrescens* or *C. atramentarium*.

From among the 20 genotypes originally surveyed in 1975, six were selected for further field evaluation in 1976. Table 1 shows the results of this investigation and provides further evidence for the validity of assaying air-dried stem tissue with the Andersen sampler. Assays from either fresh stem tissue or air-dried tissue showed similar results. When either method was used, the amount of *V. dahliae* recovered was found to be highly correlated (Table 2) with symptom expression and tuber yield. The log values of *V. dahliae* propagules recovered from assays of air-dried tissue from either stem apices or stem bases were highly correlated with isolations from fresh stem tissue ($P = 0.001$). Three potato genotypes (A66107-51, A68113-4, and Targhee) were found to be significantly more resistant than Russet Burbank to *V. dahliae*, while Nampa and Butte were significantly more susceptible. With the exception of Targhee, potato genotypes showing resistance to *V. dahliae* produced significantly higher yields. Potato grade was closely related to wilt severity and to *V. dahliae* colonization in air-dried stem tissue. All potato genotypes that demonstrated resistance also showed significant increases over Nampa, Butte, and Russet Burbank in the percentage of U.S. No. 1 potatoes.

Field results of 1977 and 1978 (Table 3) produced similar results. Relative relationships of *V. dahliae* colonization using the Andersen sampler procedure remained the same, while incidences of wilt severity and yield were also similar.

Field results from Egin Bench further substantiated the validity of *V. dahliae* assays from air-dried potato tissue by demonstrating a straight-line relationship between recovery and time. Figure 1 shows this linear relationship. In this study the time of sampling was highly correlated ($r = 0.88$, significant at $P = 0.001$) with the log values of *V. dahliae* propagules per gram of air-dried stem tissue.

In this field, *V. dahliae* propagules in the soil were also highly correlated with both wilt severity and with rate of *V. dahliae* colonization in potato-stem tissue (slope for increase of *V. dahliae* colonization). Figure 2 demonstrates this relationship. Similarly, the log values of *V. dahliae* colonization from stems collected on 17 August were highly correlated ($r = 0.70$, significant at $P = 0.01$) with log values of *V. dahliae* propagules in soil.

Table 4 provides results from a greenhouse study involving some of the same potato genotypes evaluated in the field. Initial symptom development (23 days after inoculation) corroborated results of field investigations by showing Russet Burbank and Butte cultivars to be significantly more susceptible to wilt than either Targhee, A66107-51, or A68113-4. With time, however, this relationship was found to change, and 36 days after inoculation, Butte and Targhee exhibited the most severe symptoms. Similarly, assays of air-dried petioles (collected 34 days from the time of inoculation) indicated that Butte and Targhee had significantly higher *V. dahliae* counts in tissue than either Russet Burbank, A66107-51, or A68113-4. With increased time (44 days from time of inoculation), the differences of symptom expression between Butte, Russet Burbank, and Targhee were not significant, whereas A66107-51 and A68113-4 continued to demonstrate significantly fewer symptoms.

As potatoes were shown to be less susceptible to *V. dahliae*, the relative loss of yield was also smaller. At harvest, the ratios of tuber weights from inoculated plants to the mean tuber weights of noninoculated controls continued to show this same relationship.

Table 5 demonstrates the linear correlations of *V. dahliae* recovery from air-dried tissue with wilt and yield data. The determination of *V. dahliae* colonization by the Andersen sampler method was consistently shown to be highly correlated with these factors.

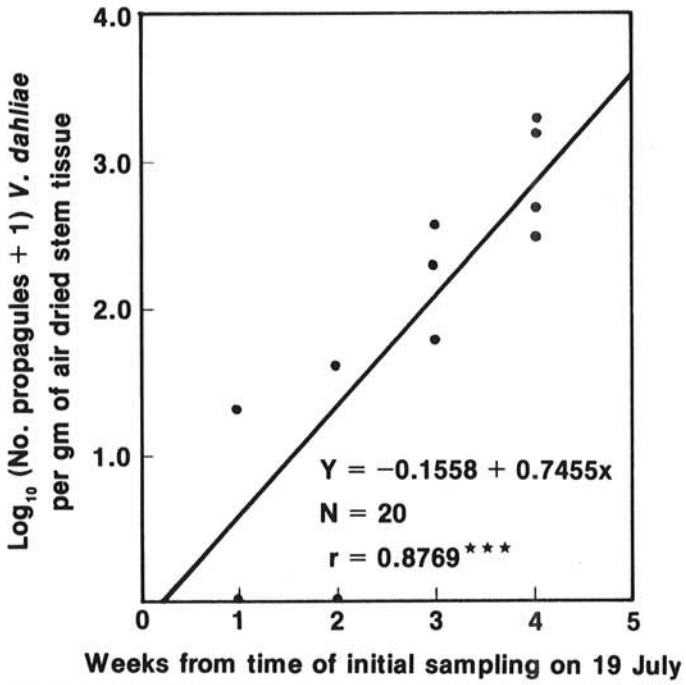

Fig. 1. The relation of time of sampling to *Verticillium dahliae* colonization in potato stem tissue in 1979.

DISCUSSION

To understand the relationships that commonly occur between *Verticillium* invasion and potato production, there is a need to quantify the colonization of pathogens within host tissue. For studies involving *V. dahliae*, it is believed that assays from air-dried stem tissue as described herein may provide a valuable tool to accomplish this objective. This method offers the advantage of making collections in the summer and assaying for *V. dahliae* colonization at a later date following harvest.

Several lines of evidence from field and greenhouse studies support the reliability of this procedure. A straight-line relationship between *V. dahliae* colonization and the time was demonstrated by the assay method. The degree of *V. dahliae* colonization within stem tissue has been consistently shown to increase with the advance of the growing season and with an increase of symptom development. Throughout these investigations, the presence of more than 100 *V. dahliae* propagules per gram of air-dried apical stem tissue has been associated with the occurrence of apical symptoms. This appears to approximate the threshold of measurable colonization that is required for symptom development of the Russet Burbank potato.

The close relationships that have been found to exist between inoculum in the soil and either wilt severity or stem colonization by *V. dahliae* lend further support to the reliability of this procedure. The recovery of *V. dahliae* from air-dried stem tissue has correlated significantly ($P = 0.01$) with soilborne inoculum. Similarly, the rate of *V. dahliae* colonization in potato stem tissue was shown to be linearly related to inoculum in the soil.

A comparison of assays from air-dried stem tissue with isolations from freshly collected plant material has provided additional evidence to support the validity of this technique. Both assay procedures (involving either freshly collected or air-dried tissue) have consistently shown similar results. Differences in propagule counts determined by either of these methods were significant and correlated with wilt severity, yield, and grade of potato.

Greenhouse studies of *V. dahliae* colonization and wilt severity have shown that propagule counts from air-dried tissue have the same relationship as symptom development resulting from controlled inoculations. Similarly, the positive linear relationships between propagule counts from air-dried petioles and either wilt data or the ratio of yield of inoculated to noninoculated plants were found to be highly significant ($P = 0.01$). Through the determination of yield ratios, genotype variability not related to Verticillium wilt resistance can probably be largely eliminated. By this means, a reliable index depicting the relative effects of *V. dahliae* on potato yield was obtained. In addition to demonstrating the validity of this technique, the results of greenhouse studies have shown a high degree of *V. dahliae* resistance to occur in two potato clones (A66107-51 and A68113-4).

With the exception of the Targhee cultivar, greenhouse investigations have agreed closely with field studies. The fact that Targhee has shown a high degree of resistance in the field, but not in the greenhouse, can be attributed to several factors. The most likely factor is possibly related to the site of resistance (eg, roots). If resistance is related to an infection barrier in roots, it would seem reasonable that excellent resistance might be achieved in the field

TABLE 5. Correlations of *Verticillium dahliae* recovery from air-dried tissue with wilt and yield relationships in a greenhouse study

Factors correlated	Log_{10}(prop + 1)/gram[a] of *V. dahliae* in apical petioles[a] *r*-Values[b]	No. of comparisons
Percent of petioles with symptoms[c]	0.8260*	25
Severe[d] symptom expression (mean plants per pot)	0.8654*	25
Ratios of inoculated–noninoculated plants for mean tuber wts	0.7117*	25
Ratios of inoculated–noninoculated plants for mean total yield	0.7217*	25

[a] Andersen sampler technique.
[b] * = Significant relationship at $P = 0.001$.
[c] Observed 36 days from time of inoculation.
[d] Severe symptom expression = stems with >75% of foliage showing evidence of symptoms 36 days from time of inoculation.

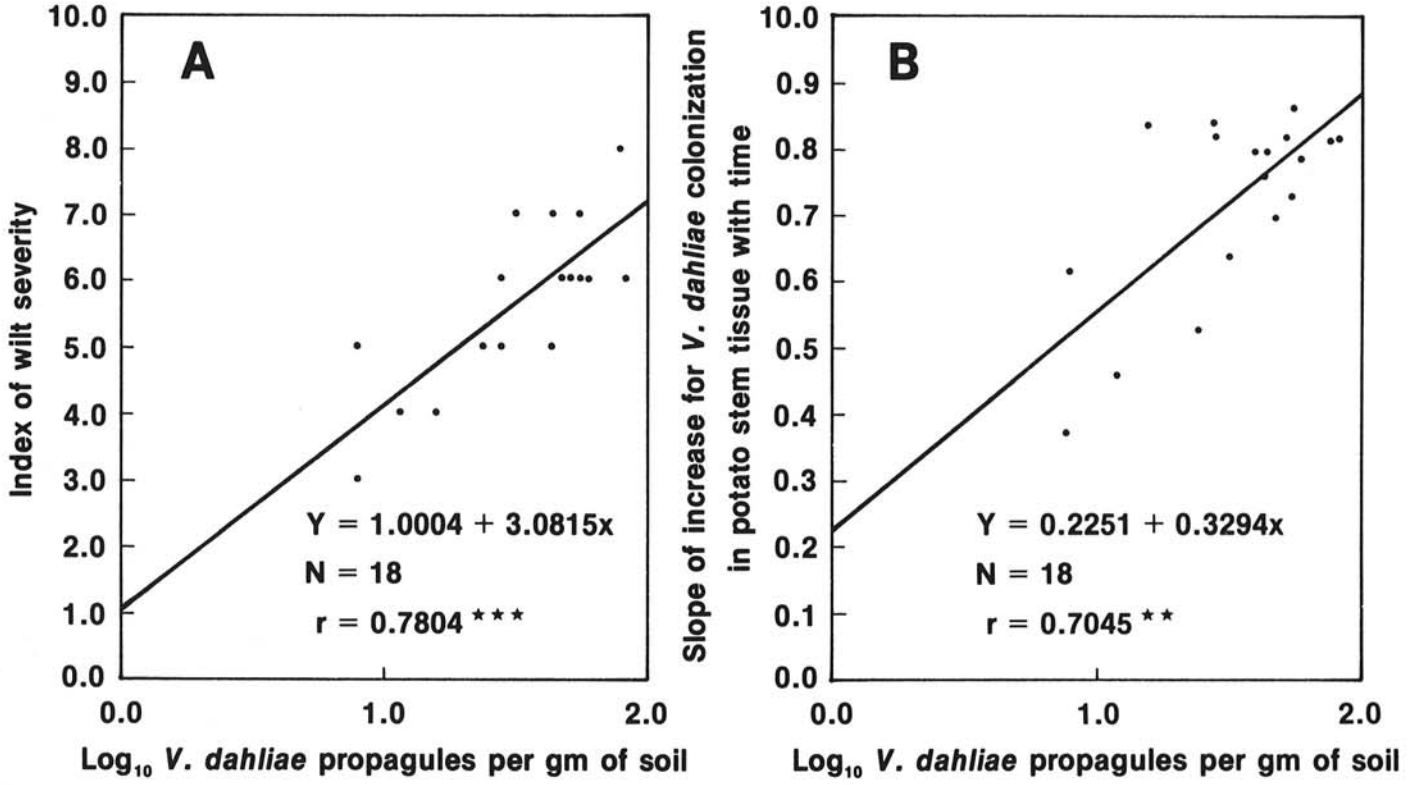

Fig. 2. The relation of *Verticillium dahliae* in soil to severity of wilt **(A)** and rate (slope) of *V. dahliae* increase in potato stem tissue **(B)** in 1979.

but not in the greenhouse since high inoculum levels were applied, and a certain amount of root injury may have occurred during the process of inoculation. This being the case, a defense barrier in roots could have been easily broken.

Results from both the greenhouse and field have provided evidence for a high degree of resistance to *V. dahliae* in certain potato genotypes. Yields of U.S. No. 1 potatoes and wilt were closely related to *V. dahliae* colonization from either the bases or apices of potato stems obtained in the field. As with greenhouse evaluations, field resistance to *V. dahliae* was closely related to increased yield. These results demonstrate the effectiveness of assays from air-dried tissue for the evaluation of relative degrees of *V. dahliae* resistance.

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