AN ABSTRACT OF THE THESIS OF

Jody J. Goodell for the degree of Master of Science

in Botany and Plant Pathology presented on August 6, 1979

Title: Potato Virus X: Detection, and Inter-relationship with

Verticillium dahliae and *Colletotrichum atramentarium*

Abstract approved: Redacted for privacy

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Preliminary studies were run to determine the sensitivity and reliability of enzyme-linked immunosorbent assay (ELISA) for the detection of potato virus X (PVX). ELISA was found to be capable of detecting PVX in tobacco at a 1:3333 sample dilution. The reliability of ELISA for the detection of PVX in potato was found to be comparable with that obtained by *Gomphrena globosa* bioassay and far superior to that obtained by greenhouse symptomatology of potato plants.

ELISA was used in a large-scale survey of PVX to determine what role, if any, PVX had on the early death of potato vines, a yield-limiting symptom-complex associated with the presence of *Verticillium dahliae*. Presence of PVX is correlated with increased severity of *V. dahliae* infection in variety 'Russet Burbank'. The probability of

random occurrence (α) of this correlation was .001. The effect of PVX on *V. dahliae* colonization was most pronounced in fields where early dying was most severe and the effect of PVX on *V. dahliae* colonization appeared to be seed-source dependent. Results suggest that the use of virus-free seed may be a useful cultural practice for reducing the level of early dying of potato vines. An inverse relationship was observed between PVX infection and the incidence and severity of *Colletotrichum atramentarium* infection, (α = .001), possibly due to an effect of the virus on plant nutritional levels.

Potato Virus X: Detection and Inter-relationship with *Verticillium dahliae* and *Colletotrichum atramentarium*

by

Jody Jellison Goodell

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Completed September 1979

Commencement June 1980

APPROVED:

Redacted for privacy

Professor of Botany and Plant Pathology
in charge of major

Redacted for privacy

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Date thesis is presented August 6, 1979

Typed by Linda S. Crooks for Jody J. Goodell

Dedicated to my parents,

Charles Albert Jellison and Phylis Gift Jellison

ACKNOWLEDGMENTS

I would like to acknowledge the help of my major professor, Dr. T. C. Allen; that of Drs. M. Powelson, R. H. Converse and H. R. Cameron of the Department of Plant Pathology, and of S. Maresh, Statistical Consulting, Oregon State University. This work was supported by a grant from the Idaho State Potato Commission.

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"IF ANY MAN HOPES TO MEET A VARIETY OF MELONS, SQUASH...OR EVEN POTATOES, WHICH UNDER NO CIRCUMSTANCES OF ILL-ADAPTED SOIL, BAD CULTURE OR INFELICITY OF WEATHER SHALL BE LIABLE TO DISEASE, HE KNOWS LITTLE OF THE WORLD IN WHICH HE LIVES."

Chauncey E. Goodrich

Country Gentleman 1863

POTATO VIRUS X: DETECTION AND INTER-RELATIONSHIPS WITH *Verticillium dahliae* and *Colletotrichum atramentarium*

GENERAL INTRODUCTION

Potato virus X (PVX), a 515 nm member of the potex group, is widely distributed wherever *Solanum tuberosum* L. is grown commercially. Symptoms in infected plants range from complete latency to mottling, curl, necrosis and severe yield reductions. Traditionally, detection of PVX has been via *Gomphrena globosa* local lesion bioassay or symptomatology of the potato plant itself. A recent report suggests that PVX can also be detected adequately by the enzyme-linked immunosorbent assay (ELISA)(107). ELISA is a quantitative serological test recently adapted from the medical profession for use in the detection of plant viruses. Its sensitivity is generally considered to be of the same order as that of the radioimmune assay and the immunofluorescence tests, but ELISA has the advantages of being a relatively safe, inexpensive test which could conceivably be used under field conditions. The form of the ELISA used in the following studies was the double-antibody ELISA, the principle of which is illustrated in Figure 1.

At present, ELISA is performed in polystyrene microplates. Specific γ-globulin is physically adsorbed to the well surfaces of the microplates. Tissue homogenate containing antigen is added to the wells, and antigen is allowed to bind immunologically to the antibody adhering to the polystyrene. Plates are rinsed to remove excess sample. Wells are then filled with antibody which has previously been conjugated to an enzyme, usually alkaline phosphatase

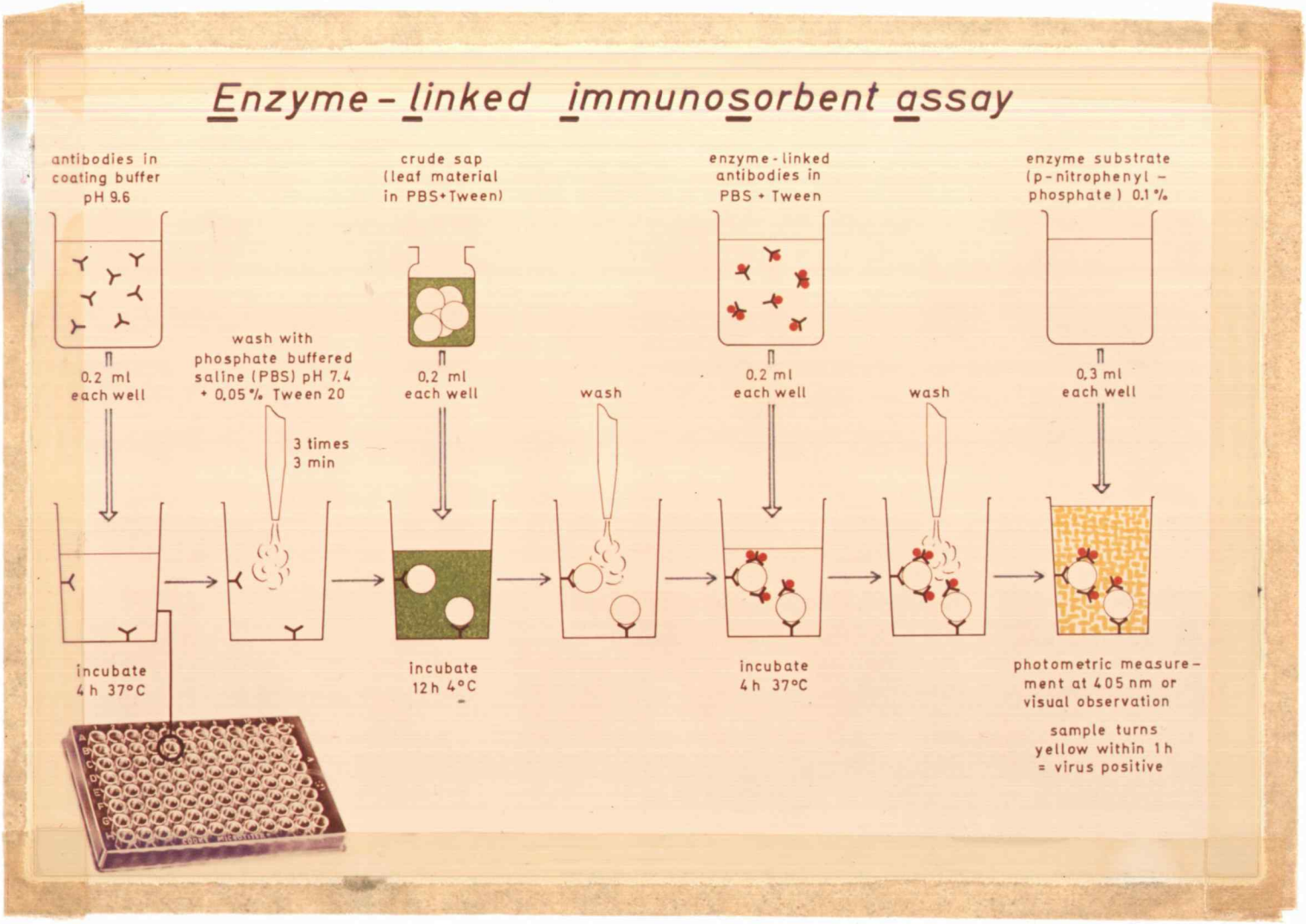


Figure 1. Principle of the Enzyme-Linked Immunosorbent Assay (ELISA, EIA).

although horse-radish peroxidase and glucose oxidase systems may also be used. The antibody end of the conjugate forms an immunological bond with the antigen which is itself bound to the solid-phase antibodies. Excess conjugate is removed by washing and an enzyme substrate is added to the wells. When alkaline phosphatase is used as the enzyme, the substrate used is p-nitrophenyl phosphate. When cleavage occurs in the presence of the phosphatase enzyme, p-nitrophenol is formed. This absorbs strongly at 405 nm in basic solution. The absorbance can be quantified spectrophotometrically and is related to the initial concentration of antigen in the tissue homogenate being tested.

In preliminary studies the sensitivity of ELISA in detecting PVX in tobacco was evaluated and the reliability of detection of mild and severe strain PVX in potato compared with that obtainable by G. globosa bioassay. The reliability of ELISA was also contrasted to the reliability of greenhouse symptomatology of potato plants in indicating the presence of mild or severe strain PVX.

The main body of this study deals with the use of ELISA in conjunction with computerized statistical techniques to evaluate the role PVX plays in early dying of potato vines. Early dying, a symptom complex characterized by premature senescence and greatly reduced yields, is a serious problem in the circle irrigation areas of the Columbia Basin of eastern Oregon. These areas, first reclaimed from the desert for agricultural use in the mid 1960's, initially produced high yields of potatoes. Continued cropping, however, has increased levels of soil-borne diseases and reduced yields.

This research was undertaken in conjunction with Dr. Mary Powelson, Vegetable Pathologist, Oregon State University. Dr. Powelson's previous work led her to postulate a primary role for Verticillium dahliae in the disease situation (96). The objectives of this study were to: evaluate the potential of ELISA for large-scale surveys; determine what effect, if any, PVX infection had upon the incidence and severity of V. dahliae infection in potato varieties Norgold Russet and Russet Burbank; examine the effect of PVX on Colletotrichum atramentarium, another fungal species abundant in fields suffering from early dying of potato vines; and evaluate indirectly the feasibility of the use of certified virus-free seed as a cultural practice for partial control of this symptom complex.

LITERATURE REVIEW

A. Potato Virus X

Reports of potato 'curl' in England date from the 1600's (65). A potato 'mosaic' was first described by Orton in 1914 who distinguished among the diseases of wilt, mosaic and leaf roll (88). Folsom claimed in 1920 that the mosaic had been present in Maine since 1912 and noted its effect on yield and physiology (40). In 1921 potato mosaic was linked to 'running out' of potatoes (the degeneration of seed stocks) by Krantz and Bisby (65). Schultz noted the ability of potato mosaic to be transmitted from one generation to the next through the tubers and hypothesized, incorrectly, that the mosaic was aphid spread (111, 112). In 1920 Schultz and Folsom showed that potato mosaic can be transmitted by "transferring juice from a diseased to a healthy" plant. This work was instrumental in implicating a "filterable virus" as the causal agent of potato mosaic (111). Edgerton and Tiebout in 1921 recommended the use of certified virus-free seed for the control of the potato mosaic, a recommendation still in force today (34). In 1924 McKay and Dykstra reported five different types of mosaic in Oregon: mild, interveinal, crinkle, rugose and leaf-rolling (75). In 1925 Gross and Peltier observed the effect of environmental conditions on the symptom expression of mosaic 'degeneration' disease (46).

The virus itself was described by Smith in 1931 and named potato virus X (PVX) (119). It has subsequently been found to be a 13 x 515 nm filamentous RNA virus (11) with a sedimentation coefficient for the whole virus at infinite dilution of 177.75, molecular weight of

35 x 10^6 daltons (100) and isoelectric point of pH 4.4. Adequate purification procedures have been developed (41). A tendency to aggregate has been reported and can be avoided by pre-adsorption to charcoal. Koenig notes that prolonged contact with crude plant sap causes degradation of the protein subunits (23). The single stranded RNA consists of about 6% of the particle weight (23) and shows molar percentages G22, A32, C24, U22. Protein composition has been reported (114, 115, 78).

Mathews, 1949, separates PVX into four groups on the basis of serology (72). Four different groups are obtained if strains are grouped on the bases of differential host infectivity (21, 22). Three still different groups are obtained if grouping is attempted on the basis of thermal inactivation points (63). The differentiation between strains is at present still subject to some disagreement but it is generally conceded that two major strain types of PVX exist, one being the latent, mild mosaic type and the other being the severe or ringspot strains (66).

Transmission has been reported by grasshoppers (134) and Synchytrium endobioticum (86) but is principally mechanical in nature. PVX is found mainly in solanaceous plants. It goes readily to tobacco and other assay hosts. Since 1948 G. globosa has been used extensively as a qualitative and quantitative diagnostic host (140, 143). The first serological investigations with plant viruses were undertaken by M. Dvorak in 1928. The use and mass production of antisera for routine testing for potato diseases began soon afterwards in Holland under the direction of van Svgteren (66). Tests commonly used for PVX detection are agglutination, precipitation and

ouchterlony gel double diffusion with virus fragments. Within the last few years a new and highly sensitive quantitative serological test has come into experimental use. This test is the enzyme-linked immunosorbent assay or ELISA.

B. Enzyme-linked Immunosorbent Assay

ELISA, which is roughly analogous to radioimmunosorbent tests, has its origins in observations made by Arvamens in 1966, that it was possible to couple an enzyme with an antibody while retaining both immunological and enzymatic activity (131). In 1967 Nakane and Pierce suggested that an indirect immuno-enzyme test might be feasible (99). In 1969 Arvameas (3) and Arvameas and Ternynck (4) published on the coupling of enzymes to proteins with glutaraldehyde and the use of the conjugates in the preparation of immunosorbents and the detection of antigens and antibodies. In 1970 Sternberger et al measured antibodies in an indirect immuno-enzyme assay (121).

ELISA in its present form was first described by Engvall and Perlmann in 1971 as a quantitative assay for immunoglobulin (35). In 1972 they published a similar paper on the quantification of antibodies by enzyme-labelled anti-immunoglobulin in antigen-coated tubes (35). Their primary contribution to the development of the test was the successful linkage of the soluble antibody to a solid phase. ELISA has since been used for the detection of hormones, serum components, snake venom, syphillis, etc. (131). Important early work includes detection of malaria using ELISA microplates (Voller et al, 1974) of Trinchinella infection in pigs, (Ruitenberg et al, 1974) of Trypanosoma cruzi (Voller et al, 1975) and detection of

antigens by ELISA (Bartlett, 1976). In September 1976 a meeting held under the auspices of the National Institute of Health at Bethesda, Maryland, examined the use of ELISA for the detection of infectious disease.

ELISA was first used specifically for the detection of plant viruses by Voller et al in 1976 (130). Much of the early work in adapting the ELISA system for use in the detection of plant virus was done by Clark and Adams (17, 18). During 1977 and 1978 reports of detection of viruses in potato by ELISA ensued: e.g., potato leaf roll (14) potato virus A, potato virus Y (68) and tobacco rattle virus (48). Salazar reported that by mixing globulins he could simultaneously detect Andean potato mottle virus, Andean potato latent virus and potato viruses X, S, V and T (107,107). A comprehensive review of ELISA, with emphasis on its medical applications, is given by Voller, Bidwell, and Bartlett (131).

C. Early Dying of Potato Vines

Early dying has previously been identified with *Verticillium* wilt (49, 97). The relationship of wilt in potatoes to inoculum potential of *Verticillium* and to the survival of *Verticillium* in the soil is well established (71, 127, 57). There are, however, indications that early dying of potato vines is the result of a disease-complex rather than a single pathogen (53). Several cases of interactions with *Verticillium dahliae* in other crops have been noted: e.g., interactions between *Fusarium oxysporum* f. *vasinfectum* and *V. dahliae* in cotton (1); cross-protection by *Verticillium nigrescens* against *V. dahliae* in mint (76); antagonism from saprophytic fungi (44); and

antagonism from certain actinomycetes towards Verticillium in cotton (114). Roles have been suggested for Colletotrichum, Fusarium and Erwinia in the causal complex of early dying (96).

Fusarium is capable of acting as a wilt pathogen even when it is not accompanied by Verticillium. The role which Colletotrichum atramentarium plays is somewhat more obscure, and though often considered primarily saprophytic C. atramentarium has been linked to "Black Dot" of potato since 1926 (30). Subsequent reports (80, 124, 121, 64) suggested that C. atramentarium is capable of a higher level of pathogenicity than previously expected. Davis in 1976 called attention to a possible relationship between C. atramentarium and Verticillium (28). Gudmestad in a 1977 North Dakota wilt survey reported a high incidence of coinfection of Verticillium and Colletotrichum (47). Otazu et al in 1978 further examined the role of Colletotrichum in the potato wilt complex prevalent in North Dakota, C. atramentarium was obtained from both wilted and non-wilted stems. Although C. atramentarium was found primarily in older and wilted stems, pathogenicity tests failed to establish a relationship between C. atramentarium and V. dahliae (89).

D. Fungal-Viral Interactions

Effects on fungal disease by viruses have previously been reported: In 1964 Farley and Lockwood reported increased susceptibility to root rots in virus-infected peas (29). In 1972 Beniwal and Gudauskas observed that virus-infected corn and sorghum were more susceptible to fungal attack (38). Mullen and Bateman reported in

1975 an effect of PVX on the enzymatic degradation of potato cell walls by Fusarium roseum avenaceum (8). In 1976 Thanassoulopoulos noted that the symptom expression of Verticillium and Fusarium in tomatoes was effected by the presence of tobacco mosaic virus (81). The pronounced effect of plant nutritional levels on Verticillium wilt (123) suggested a possible role for PVX via an effect on plant nutrition. The most extensive studies on fungal-viral interaction have been done with PVX and deal with the relationship of PVX to Phytophthora infestans, causal agent of late blight of potatoes. Muller and Munro reported in 1951 the retardation of growth of P. infestans by prior inoculation with PVX and noted a decrease in fungal sporulation as systemic PVX symptoms increased in severity (82). In 1973 Dowley noted that prior PVX infection increased field infection by P. infestans (31). De Cutillos and Thurston, 1975, did laboratory and field studies on the potato cultivar 'Katahdin' and found smaller lesions and decreased P. infestans sporulation on virus-infected plants. Pietkiewicz (26) failed to see a PVX-induced reduction in blight lesion size but did suggest that in leaves infected with PVX the liberation of zoospores may be inhibited (93, 94). Hodgson, Munro, Singh and Wood (55, 117,141) have isolated a polysaccharide from P. infestans, which apparently inhibits PVX infectivity in tobacco without reducing viral multiplication and suggests that virus entry is inhibited by a polysaccharide-mediated modification of infectible sites at the leaf surface.

PRELIMINARY STUDIES USING ELISA FOR THE DETECTION OF PVX

A. Sensitivity of ELISA

The objective of the following study was to confirm ELISA as a highly sensitive test for PVX and to establish limits to that sensitivity.

1. Materials and Methods

a. General ELISA procedure: (For a complete list of materials used and buffer compositions see Appendices i and ii.)

i. Purification of Coating γ-globulin

One ml of antisera was diluted in nine ml of distilled water. Ten ml of saturated ammonium sulfate was added a drop at a time to the above. After 30 minutes, the suspension was centrifuged at 6000 rpm. The pellet was saved and dissolved in two ml of 1/2 strength PBS buffer. This solution was then dialized three times against 500 ml portions of 1/2 strength PBS, at least once overnight. DE22 cellulose was precycled in 0.5 N HCl and 0.5 N NaOH, degassed and equilibrated with a full strength PBS buffer solution followed by equilibration with 1/2 strength PBS to pH 7.0. Fines were removed and a ten mm diameter column packed to approximately ten cm. Globulin was washed through the column with 1/2 strength PBS buffer. Effluent was monitored at 280 nm and the first protein peak collected. Concentration of the protein fraction was adjusted to approximately one mg/ml (OD 1.4) by dilution with 1/2 strength PBS or concentration by solid dialysis against polyvinyl-pyrrolidone M.W. 40.

ii. Conjugation of Alkaline Phosphatase with γ-globulin

Two mg of Sigma No. P-4502 alkaline phosphatase type VII were centrifuged at 6000 rpm for five minutes and the pellet obtained dissolved in one ml of γ-globulin and dialyzed against three changes of 500 ml PBS, at least once overnight. Electron microscope grade glutaraldehyde was added to a final concentration of 0.005% and the globulin-phosphatase mixture left at room temperature for four hours then dialyzed against three changes of 500 ml PBS plus 0.01% sodium azide. Bovine serum albumin was added to a final concentration of five mg/ml. Storage was in siliconated tubes at 5° C.

iii. Sample Testing

Two-hundred ul purified γ-globulin diluted 1:800 in coating buffer was pipeted into the inner wells of a Dynatech 1-223-29 flat bottom microtitre plate. Outer wells were filled with buffer. Plates were covered with plastic wrap and incubated at 37° C for four hours, and then overnight at 5° C.

The plate was emptied and washed thoroughly with a PBS solution to which Tween 20 has been added to 0.05%. The plate was left filled with PBS-Tween for three minutes. Washing and soaking was repeated three times. After the final soaking the plate was emptied.

Samples were ground in PBS buffer and filtered to remove debris. Two-hundred ul of the tissue homogenate, diluted appropriately for the system being used, were added per well to duplicate wells of the precoated plate. Healthy and diseased controls were included on each plate. Only inner wells were

used. Outer wells were filled with buffer. The plate was incubated overnight at 5° C.

The plate was washed as outlined above. Two-hundred ul per well of conjugated γ-globulin-alkaline phosphatase diluted 1:800 in PBS-Tween PVP egg albumin was then added. Incubation was for four hours at 37° C. In the last step the plate was washed as outlined above and 300 ul per well of freshly-prepared p-nitrophenol phosphate one mg/ml in substrate buffer added. The enzyme reaction was allowed to proceed 30 to 60 minutes at room temperature, then slowed while still in the linear phase by the addition of 50 ul per well of 3 N NaOH. Visual evaluation was done against a white background or a GB 11-8 lightbox on a six-point scale, with zero corresponding to complete lack of color and six signifying a very intense yellow. Absorbance was read at 405 nm on a Beckman Model 25 spectrophotometer.

b. Materials used in the following investigation:

ELISA was performed as outlined above with anti-PVX rabbit γ-globulin prepared by R. Casper and obtained from H. R. Converse. Coating and conjugate were diluted with coating buffer and PBS buffer respectively at a 1:800 ratio. A sample dilution series of PVX-infected and virus-free Nicotiana tobacci leaves was run from 1:1 weight to volume in PBS buffer to 1:3333 in half log steps. An overnight sample incubation at 5° C was compared with a 1.5 hour incubation of the sample at 37° C.

2. Results and Discussion

Results are presented in Figure 2. The shape of the dilution-absorbency curve is similar for the overnight 5° C sample incubation and the 1.5 hour, 37° C sample incubation. Sensitivity is adequate to a 1:3000 sample dilution for both incubation procedures. Though intra-plate variation between duplicate samples was greater using the 1.5 hour 37° C sample incubation, this disadvantage was outweighed by the significantly superior sensitivity obtained using the shorter, hotter, incubation which apparently favored the quickly forming immunological bonds while not greatly increasing the level of non-specific binding of sample proteins to exposed polystyrene walls. Figure 3 shows the absorbance at 405 nm as influenced by the log of the sample dilution factor. Note that the transformation to a log scale has not resulted in a linear function.

The optimum coating and conjugate concentrations and incubation times will vary depending upon the plant part or type being assayed and should be established independently for each globulin and plant type tested.

Sensitivity can be further increased by increasing the volume of coating buffer plus globulin per well from 200 ul to 225 ul. This tends to decrease plate background level, possibly by reducing the chances of the conjugate binding directly to exposed non-coated polystyrene wall surface. A step-wise ammonium sulfate precipitation may be substituted for a one-step precipitation of the

Figure 2. Dilution series of PVX-infected tobacco and the effect of sample incubation time and temperature on the sensitivity of ELISA. Graph represents the mean of eight replicates. Buffer background has been subtracted.
A1 = Virus positive sample incubated 1½ hours at 37° C.
A2 = Virus negative control incubated 1½ hours at 37° C.
B1 = Virus positive sample incubated overnight at 5° C.
B2 = Virus negative control incubated overnight at 5° C.

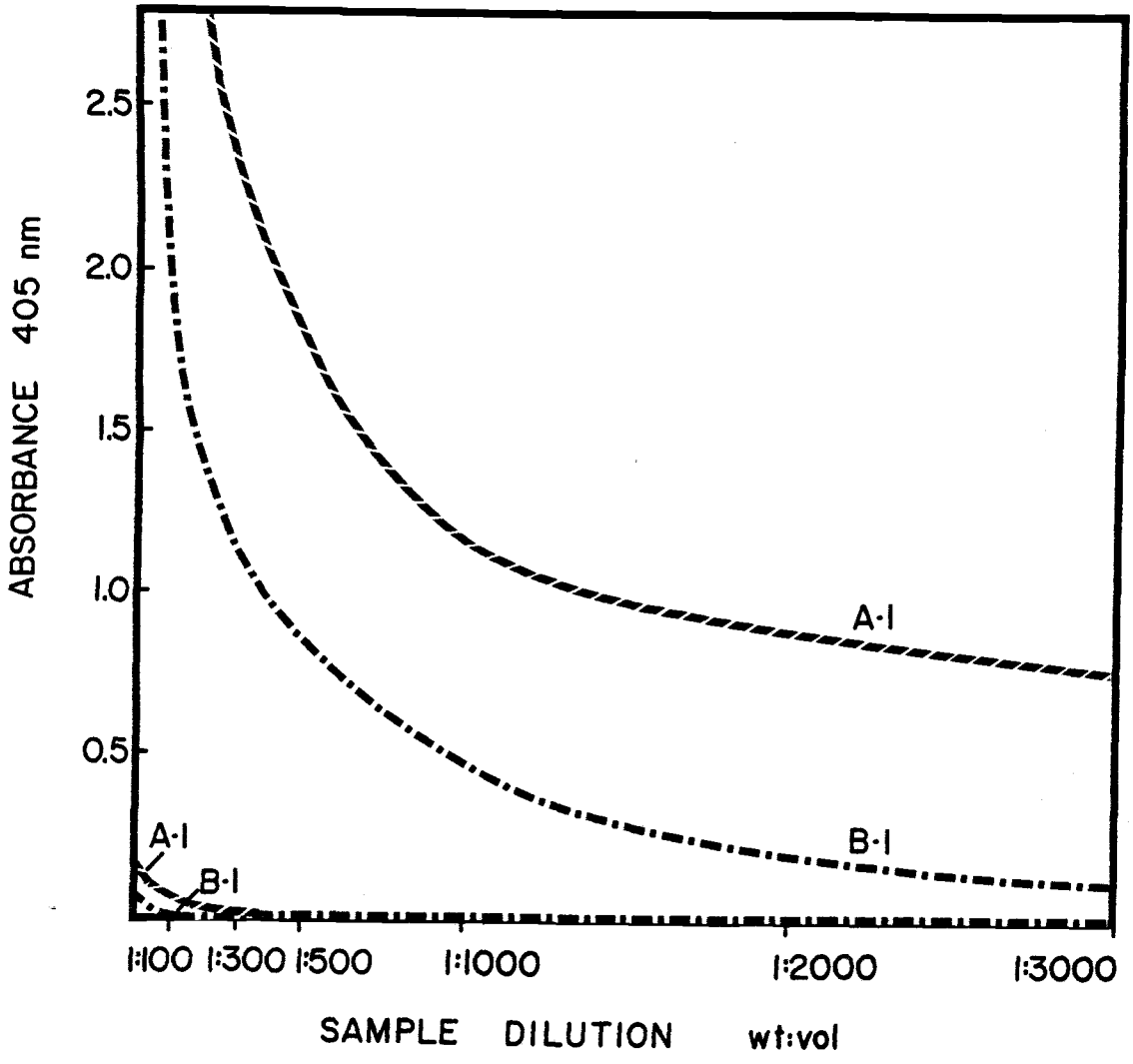
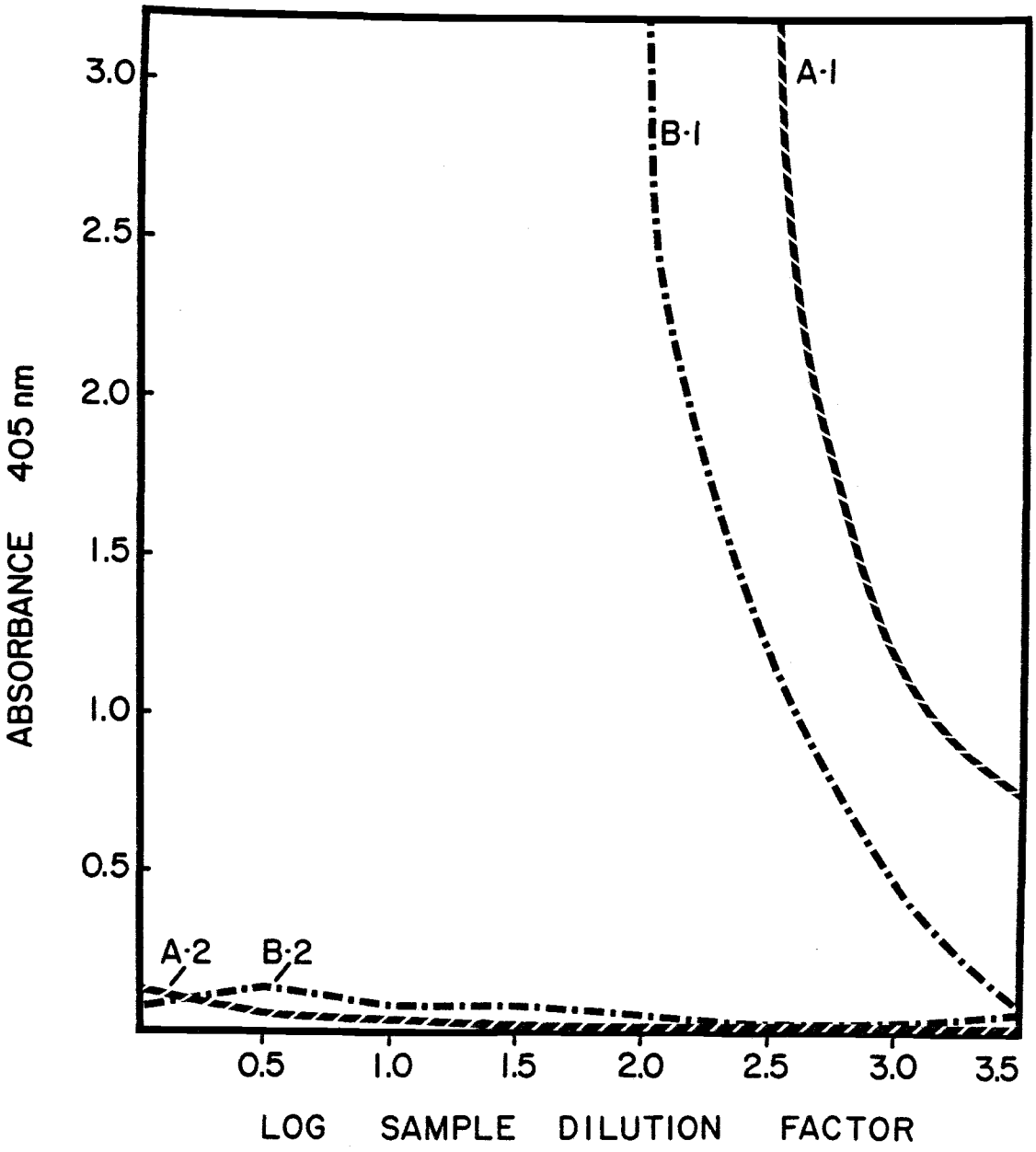

ABSORBANCE 405 nm
2.5
2.0
1.5
1.0
0.5
A-1
B-1
A-1
B-1
1:100
1:300
1:500
1:1000
1:2000
1:3000
SAMPLE DILUTION wt:vol

Figure 3. Log dilution series of PVX infected tobacco and the effect of sample incubation times and temperature on the sensitivity of ELISA. Graph represents the mean of eight replicates. Buffer background has been subtracted.
A1 = Virus positive sample incubated 1½ hours at 37° C.
A2 = Virus negative control incubated 1½ hours at 37° C.
B1 = Virus positive sample incubated overnight at 5° C.
B2 = Virus negative control incubated overnight at 5° C.


3.0
2.5
2.0
1.5
1.0
0.5
ABSORBANCE 405 nm
A-1
B-1
A-2
B-2
0.5
1.0
1.5
2.0
2.5
3.0
3.5
LOG SAMPLE DILUTION FACTOR

globulin.* The advantage of step-wise precipitation is that it provides a greater yield of a more finely purified globulin. Ultimately the sensitivity of ELISA is dependent upon the specificity and purity of the globulin used. When relatively non-specific globulin must be used, greater specificity can be obtained by pre-adsorption with healthy plant material. When globulin is contaminated by proteases, hemoglobin or other blood components, it can be purified by additional precipitation or column filtration steps.

3. Conclusions

A curvilinear relationship exists between the initial PVX concentration in the sample being tested and the A_{405} obtained using the ELISA test. ELISA was able to distinguish between healthy and PVX-infected tobacco leaves to a dilution of 1:3000. The sensitivity of ELISA is increased through the use of short, 37° C sample and conjugate incubations.

B. Reliability of ELISA

Field studies carried out in 1977 by Dr. T. C. Allen and P. Koepsell of Oregon State University indicated that a significant amount of PVX inoculated into Russet Burbank potato plants was escaping detection in field assays. The following study was undertaken as part of a continuing project designed to examine three

*If a step-wise precipitation is to be used, ammonium sulfate is added dropwise while stirring at 5° C until globulin is 42% saturated. The globulin-ammonium sulfate is then allowed to mix for one hour and is centrifuged ten minutes at 10,000 RPM to collect the precipitate. This procedure is then repeated, using first 38% and then 35% saturation (70).

alternate assay methods for PVX: The ELISA, Gomphrena globosa bioassay and greenhouse symptomatology. The reliability of ELISA for detection of two strains of PVX was compared with that of the standard local lesion bioassay test using G. globosa. Tubers obtained from the field study were used for greenhouse symptomatology tests to establish the relative reliability of ELISA and visual inspection of potato plants for the detection of the mild and the severe strains of PVX.

1. Materials and Methods

Twenty-eight field plots were established in a randomized block design at the Powel Butte Central Oregon Experiment Station. Plots were established as 15-foot double rows with 30 seed pieces per plot, four feet between plots and a six-foot row spacing. Seed used was variety Russet Burbank generation one, with no rod-shaped particles in electron microscope examination from Oregon State Seed Certification. A pre-planting captain dip was used. Knives were sanitized with trisodium phosphate between cuts. The plots were isolated from the rest of the field by hedge rows of non-inoculated potatoes and were watered by a fixed irrigation system. The fertilizer regime consisted of 100 lbs./acre of N, P and K obtained from a 16-16-16 commercial mix.

Seven treatments were used with four replicates per treatment. The treatments used were as follows: 1) A cutting inoculation with a mild strain PVX at planting; 2) Inoculation of plant leaves with a mild strain PVX July 2; 3) Inoculation of plant leaves with a mild strain PVX July 31; 4) Inoculation of plant leaves with a mild strain PVX August 31; 5) Inoculation of plant leaves with a mild

strain PVX July 2, followed by an inoculation with severe strain PVX on July 31; 6) Inoculation of plant leaves with a severe strain PVX, July 31; and 7) Noninoculated control.

Leaf inoculations were done with carborundum abrasive, with previously inoculated Russet Burbank leaves ground in phosphate buffer as the virus source. Approximately three leaves per potato plant were inoculated.

G. globosa versus ELISA Testing:

Sampling was done on August 1. One leaf per plant was collected. One leaflet was used for Gomphrena globosa bioassay (140). The bioassay results were considered positive if one or more lesions appeared on the inoculated G. globosa leaf. Another leaflet from the same leaf was put through an E. Pollähne automatic roller press and the resultant drop of sap diluted 1:20 in PBS buffer and used for testing by ELISA. Horse γ-globulin used was obtained from Lisse, The Netherlands, and rabbit γ-globulin prepared by R. Casper was supplied by R. Converse. The same globulin preparation was used to test both strains of PVX. ELISA was performed as outlined in section III-A-1-a. Coating globulin was 1:800 in carbonate buffer and conjugate was diluted 1:1000 in PBS buffer. Incubation times for coating, sample, conjugate and substrate respectively were four hours at 37° C and overnight at 5° C, overnight at 5° C, three hours at 37° C, and 45 minutes at room temperature. Samples were considered positive if, upon addition of substrate, duplicate wells which had previously contained sample gave OD_{405}'s which were three times greater than healthy background.

Greenhouse Symptomatology vs. ELISA Testing:

Thirty tubers were collected from each of the 28 plots established at the central Oregon field plots. Replicates and treatments were kept separate. A portion of a tuber and the eye was removed from the "blossom-end" of each tuber, using a trisodium phosphate-treated melon scoop. A numbered metal tag was inserted into each seed piece and the corresponding number used to label the remaining tuber. Each of the 840 seed pieces and corresponding tubers were labeled to enable later comparisons on a per plant basis. Seed pieces were captan-dipped and then planted in a greenhouse at a depth of one to two inches on an eight inch spacing. Potato plants were grown to a height of approximately eight inches then read, visually with symptomless plants being pulled. Plants were re-read approximately two weeks later. Greenhouse plants were considered as positives if they showed mild to severe mottling when observed in the shade. Thirty plants, both symptomless and symptomatic, were dug and potted for later ELISA testing of the leaves.

The tubers from which the seed pieces were taken were all tested using ELISA for the presence of PVX. Four eyes taken from each tuber with a number two cork borer were ground in five ml PBS buffer for testing in duplicate. ELISA was run as outlined earlier.

2. Results

a. G. globosa versus ELISA

A comparison of G. globosa bioassay results and ELISA results is shown in Table 1 and Appendix iii. With the exception of control

TABLE 1. A comparison of Gomphrena globosa bioassay and ELISA results for Russet Burbank potato leaf tests.

Treatment	Replicate	Percentage of plants diagnosed as PVX positive: Bioassay detection	ELISA detection
Control	I	0	13
	II	37	0
	III	27	27
	IV	27	10
	average	22	13
mPVX at cutting	I	57	7
	II	33	33
	III	13	3
	IV	N/A	10
	average	34	13
mPVX inoculated July 31st	I	93	73
	II	64	63
	III	100	87
	IV	59	41
	average	79	66
sPVX inoculated July 31st	I	100	77
	II	80	63
	III	77	53
	IV	69	50
	average	83	61

mPVX = mild PVX
sPVX = severe PVX

TABLE 2. Percent detection of PVX by tuber ELISA vs greenhouse symptomatology. Tuber ELISA was done using four eyes diluted in five ml PBS.

Treatment	Percentage of plants infected with PVX	
	Tuber ELISA	GH sympt.
1. mild PVX inoculated at cutting	20%	6%
2. mild PVX inoculated 7/2	98%	9%
3. mild PVX inoculated 7/31	97%	11%
4. mild PVX inoculated 9/3	55%	9%
5. mild PVX inoculated 7/2 and severe PVX inoculated 7/31	100%	15%
6. severe PVX inoculated 7/31	93%	71%
7. Uninoculated control	16%	6%

Grown from tubers from plants inoculated with severe PVX which had previously been inoculated with mild PVX.

Grown from tubers from plants inoculated with severe PVX.

Figure 4. Generalized dwarfing seen in Russet Burbanks inoculated with severe PVX and apparent cross-protection in plants previously inoculated with mild PVX.

A.

B.

Figure 5. Contrasting appearance of Russet Burbank potato plants grown from tubers harvested from plants inoculated with severe PVX (A) and from tubers harvested from plants inoculated with severe PVX after prior inoculation with mild PVX (B).

replicate #1, all of the four replicates of each of the four treatments tested showed a greater level of virus infection when assayed with G. globosa. When evaluated on a plant by plant basis agreement between G. globosa diagnosis and ELISA diagnosis ranged from 48% for replicate I of the plants inoculated with mild PVX at cutting, to 90% for replicate III of the same treatment and for replicate III of the control. An average agreement of evaluations on a per plant basis was 66% for mild PVX inoculation at cutting, 80% for mild PVX inoculated July 31st, 70% for severe ringspot PVX inoculated July 31st, 83% for the control and 75% agreement overall on the 439 plants tested. Most of the percentage disagreement is accounted for by the lower percentage of plants diagnosed as PVX-infected using the ELISA procedure. A related study by T. C. Allen and J. Davis using Russet Burbank potatoes grown at the Idaho Experiment station during the 1978 field season shows similar results, with ELISA detection ranging from 8% higher to 18% lower than bioassay in the two replicates tested.

Three times healthy background was used as the criterion for PVX infection in this study to minimize the probability of diagnosing a virus-free plant as virus positive. When the two times healthy rule is utilized instead of only considering those which are unequivocally PVX-infected, the percentage of plants diagnosed as PVX infected is somewhat increased.

Referring back to Table 1, the average percentage of plants diagnosed as PVX-infected using the two times rule is: 13% infection for the control replicates (up 0% from that obtained using the 3X's rule); 15% for mild PVX inoculation (up 2%); 70% for mild PVX

inoculation July 31 (up 4%); and 66% for severe PVX inoculation July 31 (up 5%).

Greenhouse Symptomatology vs. ELISA:

Of the 772 plants planted in the greenhouse, 68% were positive by ELISA testing vs 18% by visual inspection (Figure 5A). Of treatments 1 through 7, only treatment 6, severe PVX inoculated July 31, showed extensive PVX symptoms in the greenhouse when tubers harvested from the trials were planted in the greenhouse. Agreement between replicates was within a few percentage points. Symptoms were seen in the other treatments in 6% to 15% of the plants, but many of these symptoms were more indicative of other viruses or disorders, such as the yellowing of lower leaves indicative of PVY. The most prominent symptom on these plants was dwarfing which could also be ascribed to non-viral causes. Severe dwarfing was seen in an average of 3.5% of all the plants with the greatest level (9%) being observed in treatment 6.

Treatment 6 was characterized by generalized dwarfing and severe mottling symptoms in 71% of the plants. Two plants showed rugose symptoms characteristic of coinfection by PVX and PVY.

Three points are worthy of note: 1) Greenhouse symptomatology is a completely inadequate method of testing for PVX and is capable of picking up only the severe PVX strain in 'Russet Burbanks'. Even severe PVX was picked up less frequently using greenhouse testing than tuber testing with tuber ELISA tests (71% vs 93%) (Table 5). Thirty randomly-selected plants were tested by leaf ELISA to make sure that the failure to detect mild PVX by greenhouse symptomatology

was not due to a failure of PVX to move from the seed piece up into the plant. It was found in all cases that the virus had moved up into the plant. 2) ELISA is incapable of distinguishing between the mild and severe strains of PVX. Greenhouse testing is apparently an effective way of distinguishing between severe and mild strain PVX infection, though incapable of distinguishing between mild strain PVX infection and virus freedom. 3) Prior inoculation of mild PVX strain in treatment 5 followed by a later inoculation of severe PVX yields no generalized dwarfing and only a small percentage of plants displaying symptoms associated with PVX infection. This suggests that a cross-protection mechanism is taking place between the mild strain of PVX and the severe strain, and indicates that under some conditions even severe PVX diagnosis cannot be accurately made on the basis of symptomatology of the potato plant alone.

3. Conclusions

Using the criteria used in this study to qualify for virus infection, ELISA appears somewhat less reliable than the standard *Gomphrena globosa* test for detection of PVX in potato leaves. The apparent reliability of ELISA can be increased by using the two times healthy background rule to indicate virus infection, but with a concurrent increase in the probability of inaccurately diagnosing a healthy plant as virus-infected. It should be noted, however, that the response of the *G. globosa* assay is dependent upon many variables such as the light and temperature regimen under which the plants are grown (140) and is itself subject to error. Ideally, dual testing using both ELISA and *Gomphrena globosa* should be employed.

ELISA appears able to detect both mild and severe strains of PVX adequately. This is in keeping with previous observations (66) that the strains of PVX are closely related serologically. The relative levels of detection of mild and severe PVX strains which are obtainable with ELISA have not, however, been rigorously tested. The work of R. Koenig (62), distinguishing between homologous and heterologous strain reactions and noting an increased serological specificity obtained at the conjugation step, suggests that caution should be used when antibodies made from a specific PVX strain are used to test for other strains.

Greenhouse symptomatology in potatoes is not an adequate test for the detection of mild PVX infection, but can be used to distinguish between mild and severe strains of PVX. Cross-protection is apparently occurring between mild PVX and severe PVX and should be taken into consideration when evaluating the advisability of using greenhouse symptomatology in testing for severe PVX infection.

Problems remain in the utilization of ELISA. The extreme sensitivity of the test makes it highly vulnerable to contamination and imprecision. Non-specific binding of healthy plant compounds may also be a problem, especially when working with a high-coating globulin concentration or plant materials with a high concentration of interfering species such as phenols. Absolute quantification of unpurified PVX by ELISA is difficult. The results of the above-mentioned preliminary studies on sensitivity and reliability, however, indicate a high potential for the use of ELISA.

INTER-RELATIONSHIPS AMONG PVX, VERTICILLIUM DAHLIAE AND COLLETOTRICHUM ATRAMENTARIUM IN NORGOLD RUSSET AND RUSSET BURBANK POTATOES

Early dying of potato vines is characterized by premature senescence and has been associated with the presence of Verticillium dahliae (49, 97). There are, however, indications that early dying is the result of a disease complex rather than the result of a single pathogen (53). Observations that a vigorous plant is often a Verticillium resistant plant (29) and the deleterious effect of Potato Virus X (PVX) on plant vigor levels suggested a possible role for PVX in the early dying complex.

PVX levels were monitored and analyzed to determine if the level of virus infection in two fields was capable of influencing the V. dahliae levels and indirectly the severity of early dying of potato vines. Levels of Colletotrichum atramentarium were also monitored to ascertain what role the 'black dot' pathogen played in early dying and what effect, if any, PVX had upon the level of C. atramentarium colonization.

A. Materials and Methods

1. Field Procedures

Sixty-four plots were established in two center-pivot circle irrigation areas in the Columbia River Basin of Eastern Oregon in 1978. One of these circles was new to potato production and was selected to serve as a location for control plots because soil-borne diseases are generally not a limiting factor in first year circles. The second circle had been in potato production for four years of the past five

years with winter wheat as a rotation crop in 1976. In 1977, one year before the initiation of this study, early dying sumptoms had been apparent in 95% of the potato plants, cv. 'Russet Burbank' in this circle.

Three different seed lot sources of potato, Solanum tuberosum L. cv. 'Russet Burbank' and one source of 'Norgold Russet' were used in this study. The 'Russet Burbank' sources originated from Oregon, Montana and Idaho whereas Nebraska was the source of the 'Norgold Russet' seed lot. Each seed tuber was washed in tap water and surface sterilized for three min. in a 5% commercial bleach solution to eliminate any surface-borne inoculum of V. dahliae. The stem end was removed and the remaining blossom end cut in half longitudinally so that two somewhat bilaterally symmetrical seed pieces weighing 42 to 57 g. were obtained. Sister seed pieces were kept separate. Each seed piece was suberized at 13° C for six days.

Two treatments, seed pieces inoculated with microsclerotia and conidia of V. dahliae and non-inoculated seed pieces were used to insure some infection in the field new to potato production. Czapek Dox agar plates were seeded with conidia of V. dahliae and incubated at 22° C for one month. Conidia and microsclerotia were harvested by grinding the seeded agar in a 0.1% methylcellulose solution for one minute and then filtered through several layers of cheese cloth. The inoculum level was adjusted using water to 1400 microsclerotia per ml of solution. The inoculum was applied to all surfaces of one half of the sister seed pieces using a paint sprayer. The other half of each seed piece was sprayed with 0.1% methylcellulose solution to serve as a control.

Plots were established April 14, 1978 in a randomized block design with four replications at each of the two locations. One half of each seed piece was planted at each location at a seeding rate of 515 kg/ha. Each treatment (seed lot) consisted of two six-m rows on an 86.4 cm row spacing. Inoculated and uninoculated seed pieces were the main treatments at each location with seed lots the subplots. Fertilizer and pesticides were applied by the growers.

Two weeks prior to plot harvest, 25 plants from each of the 64 plots (two locations x two treatments x four seed sources x four replications) were selected at random and brought back to the laboratory. A fresh stem segment, about six cm in length, was taken at the soil line and was washed in running tap water and then surface sterilized in a 5% commercial bleach solution for three min. The epidermis was removed and a two cm stem segment cut off. Each section was chopped into ca. 5-6 segments and these segments were then macerated in an Omni-Mixer homogenizing chamber at 10,000 rev./min. for one min. in 50 ml of a one molal phosphate buffered saline (PBS) solution to which 0.2% egg albumin and 2% polyvinyl pyrrolidone 40 (PVP 40) had been added.

2. PVX Quantification

Tissue homogenates were tested for PVX by ELISA with horse γ-globulin obtained from Lisse, The Netherlands. Purification of the globulin was via stepwise ammonium sulfate precipitation and subsequent passage through a DEAE column as described earlier. Globulin was concentrated by solid dialysis with PVP 40 to an A_{280} of 1.4 and diluted 1:800 in carbonate buffer for coating of plates. Plates

were incubated four hours at 37° C and overnight at 5° C with 225 ul coating buffer. Plates were emptied and rinsed with running tap water before use. Samples of potato stems were ground in PBS as noted above and filtered through kimiwipes to remove debris. Two hundred ul of the filtered sample homogenate was added to coated plates. Each sample was tested in duplicate with appropriate controls on each plate. Five or six samples from each plate were retested on an additional plate to obtain a measure of interplate variability. After an overnight incubation at 5° C or a four hour incubation at 37° C, plates were emptied and rinsed. γ-globulin-phosphatase conjugate was added in PBS-PVP at a dilution of 1:1000 200 ul/well. Plates were incubated at 37° C for three to four hours and then emptied and rinsed. P-nitrophenol phosphate in 40 ml substrate buffer pH 9.8 was added at 300 ul/well and incubated at room temperature for 30-60 minutes. The reaction was slowed with 25 ul 6N NaOH/well and then read visually and with a Bechman Model 25 spectrophotometer at 405 nm.

3. Fungal Quantification

The same tissue homogenates which were used for viral quantification were also used for fungal quantification. Five ml aliquots were pipeted into warm (44° C) ethanol-water agar containing 100 ppm streptomycin sulfate and 50 ppm each of chlorotetracycline and chloramphenicol (84). The mixture was shaken and then poured into five Petri dishes and incubated in the dark for two to three weeks. Colonies of V. dahliae and C. atramentarium were counted and number of units of V. dahliae and C. atramentarium/cm stem tissue were

determined. This was used as a measure of severity of colonization of the xylem tissue and also served as a means of determining incidence of both pathogens.

4. Data Analysis

The absorbency readings obtained from the ELISA tests were converted into an artificial rating as follows: 0 = very low A, background level, indicative of virus freedom; 1 = medium A, above background but indicative of relatively low virus level as from a late season infection; 2 = high A indicative of a high virus concentration present in the test sample as might be expected from seed piece or early season infection. Ratings of groups 1 and 2 were combined as a virus-positive group or Rating 2 was used alone and designated as a virus-high group. The necessity for reducing the data from A readings to qualitative groups arises from: the semi-quantitative manner in which the samples were prepared; i.e., on a constant stem segment length basis rather than weight/volume basis; the difficulty that statistically each plate must be treated as a discrete sample unless interplate variation can be reduced to the level of intra-plate variation, or unless individual plate readings are converted to rankings or ratios; and the fact that the strain specificity of PVX has not been adequately determined for ELISA (62). Because of the qualitative, non-continuous nature of the groupings, analysis was in terms of point-biserial correlation instead of T-tests, and such analyses as stepwise-multiple regression were only used in instances where the virus ratings could be converted to a quantitative indicator, such as percent virus infection per plot.

Probability evaluations were made using natural log of the vascular colonization ratings instead of the raw data. In addition, by analyzing on the basis of natural logs, excess variation due to errors in the counting process could be reduced. All analyses, however, were repeated using the untransformed data to make sure that the observed relationships were not a product of data manipulation.

B. Results and Discussion

The raw data obtained in this study has been stored on magnetic tape and is available upon request from the author or Dr. T. C. Allen, Dept. Botany and Plant Pathology, Oregon State University.

A comparison of visual and spectrophotometric analysis of ELISA is presented in Appendix iv.

1. Distribution of PVX, Verticillium dahliae and Colletotrichum atramentarium

PVX was not uniformly distributed throughout the plots or seed sources. When the percent PVX infection per plot was averaged for the individual seed sources, a considerable variation was observed. With seed sources 1 and 3 of the variety 'Russet Burbank' 76.4% and 68.3% were PVX infected respectively. Seed source 2, also of the variety 'Russet Burbank' showed a significantly lower level of virus infection with an average percentage of PVX infection of 42.8%. The lowest level of PVX infection was observed in seed source 4 (Norgold Russet) which had an average of 33.4% infection. The same patterns of percent virus infection per seed source were observed if locations were partitioned out, however, PVX infection was consistently higher in plants sampled

TABLE 3. Incidence of PVX in three Russet Burbank seed lots (1, 2, 3) and one Norgold Russet seed lot (4) grown at two locations in the Columbia Basin of eastern Oregon. Location 1 represents a field new to potato production. Location 2 represents an old field.

Seed source	Location	% PVX infection	Ave. % PVX per seed source
1	1	82.2	76.4
1	2	70.6	
2	1	46.1	42.8
2	2	39.5	
3	1	74.3	68.3
3	2	62.4	
4	1	37.5	33.4
4	2	29.4	

from the new field (Table 3). Presumably this was due to differences in the rapidity of PVX spread due to slightly different cultural practices at the two locations.

An analysis of variance confirmed the above observation of a dependence of PVX incidence upon seed source and location. The F value for treatment (representing V. dahliae inoculated plots vs. non-inoculated plots) is very low indicating that PVX incidence is independent of V. dahliae incidence, i.e., any correlation between PVX and V. dahliae is a result of a dependence of V. dahliae upon prior PVX-infection and not vice versa.

2. Effect of PVX on Incidence and Colonization by Verticillium dahliae

When the mean number of V. dahliae units per cm stem in virus free plants is compared with PVX positive plants, a higher level of V. dahliae is found in the virus positive plants.

Table 4. T-test of the mean Verticillium dahliae values (units/cm stem) for virus positive and virus free plants. Verticillium dahliae means were obtained by averaging colonization levels of both Verticillium dahliae infected and Verticillium dahliae free plants.

	# Cases	Mean	P_{rb}	T	2-tail prob.
Virus positive	807	6529.15	0.0411	1.53	.1250
Virus free	587	5446.40			

Testing significance using point-biseriate correlations (P_{rb}), a random probability of 0.1250 is found. P_{rb} was used instead of the normal methods of calculating T values due to the non-quantitative nature of group divisions. (Equations 1 and 2)

$$*P_{rb} = (A - B)\frac{C \times D^{\frac{1}{2}}}{E^2} \qquad T = \frac{(-R_{pb})\ E}{1 - (R_{pb}{}^2)} \qquad \text{Equation 1}$$

2-tail prob from *CRTDIS fortran program on Oregon State 3

A = mean 1 C = # in Group 1

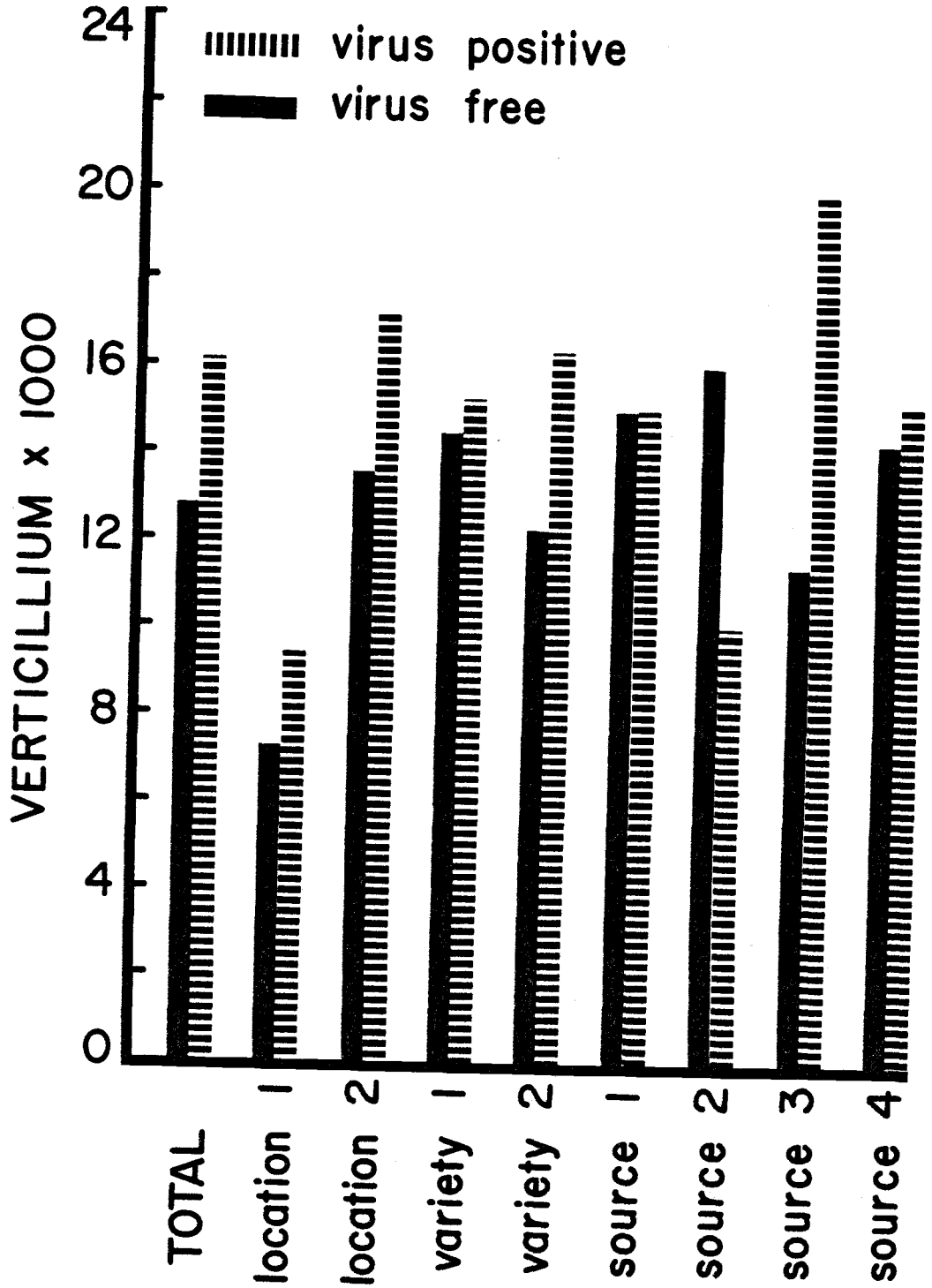
B = mean 2 D = # in Group 2 Equation 2

E = s of combined groups

The high random probability level indicates a lack of significance, but examining the raw means and partitioning by location, variety, seed source and treatment (Figure 6), a relatively consistent pattern is observed. With the exception of seed source 2, all locations, sources, and treatments had a higher V. dahliae level in the presence of PVX. This effect is particularly pronounced in the old circle where early dying was severe. Treatments 1 (non-inoculated seed pieces) and 2 (seed pieces inoculated with V. dahliae) did not significantly affect the level of PVX nor did the general effect which PVX had upon the observed level of V. dahliae colonization differ between the two treatments. In both treatments a higher mean colonization of V. dahliae was observed in PVX infected plants.

V. dahliae levels were calculated by averaging the number of propagules per cm at stem base over the total number of plants regardless of whether they were infected by V. dahliae or not. Consequently, the means obtained are a function of both Verticillium infected and noninfected plants. Two modeling systems were used; one contrasting the means in PVX positive vs. PVX free plants (Figure 6), the other contrasting the means of PVX free plants with

Figure 6. Mean Verticillium dahliae propagules per cm stem tissue averaged over Verticillium dahliae infected plants and shown for PVX positive and PVX free plants.
Location 1 = new field
Location 2 = old field
Variety 1 = 'Norgold Russet' seed source 4
Variety 2 = 'Russet Burbank' seed sources 1, 2, and 3
Treatment 1 = noninoculated
Treatment 2 = inoculated with Verticillium dahliae


virus positive
virus free
VERTICILLIUM x 1000
0
4
8
12
16
20
24
TOTAL
location 1
location 2
variety 1
variety 2
source 1
source 2
source 3
source 4

the means of plants which show a high level of PVX infection (Figure 7). If the relationship is valid, one would expect the relationships observed using the first system would be mirrored and perhaps amplified in the relationships observed using the second system. The data represented in Figure 7 indicates that this is in fact the case. Means for V. dahliae levels obtained upon partitioning and their individual random probability levels are given in Appendix v.

X^2 analysis comparing observed versus expected incidence frequencies of V. dahliae infected plants were run to determine if the relationship observed in Figures 6 and 7 was due to a relationship between incidence of both PVX and V. dahliae. No relationship was found as evidenced by a low X^2 (1.4). This was confirmed by linear regression of percent incidence of PVX against incidence of V. dahliae infected plants per plot which yielded a zero correlation coefficient. PVX was apparently not capable of influencing V. dahliae incidence, indicating that any effect of PVX upon V. dahliae level would have to be via an effect on V. dahliae colonization.

The effect of PVX on the V. dahliae colonization of the vascular tissue of individual plants was tested using P_{rb} and T tests with PVX-high versus PVX-free as a prediction for natural log (ln) V. dahliae colonization. A higher mean ln severity level is observed in the virus infected plants with a random probability of 0.001 (Table 6). When looking at each variety individually we see that for both the 'Norgold Russets' and the Russet Burbanks higher ln colonization levels by V. dahliae are seen in the virus infected plants. The random probability for this occurrence, however, is exceedingly low in the Russet Burbanks ($\alpha = .001$). In the 'Norgold Russets', due to a

Figure 7. Mean Verticillium dahliae propagules per cm stem tissue averaged over Verticillium dahliae infected and non-infected plants and shown for PVX-free plants and plants showing a high level of PVX infection.
Location 1 = new field
Location 2 = old field
Variety 1 = 'Norgold Russet' seed source 4
Variety 2 = 'Russet Burbank' seed sources 1, 2, and 3
Treatment 1 = noninoculated
Treatment 2 = inoculated with Verticillium dahliae

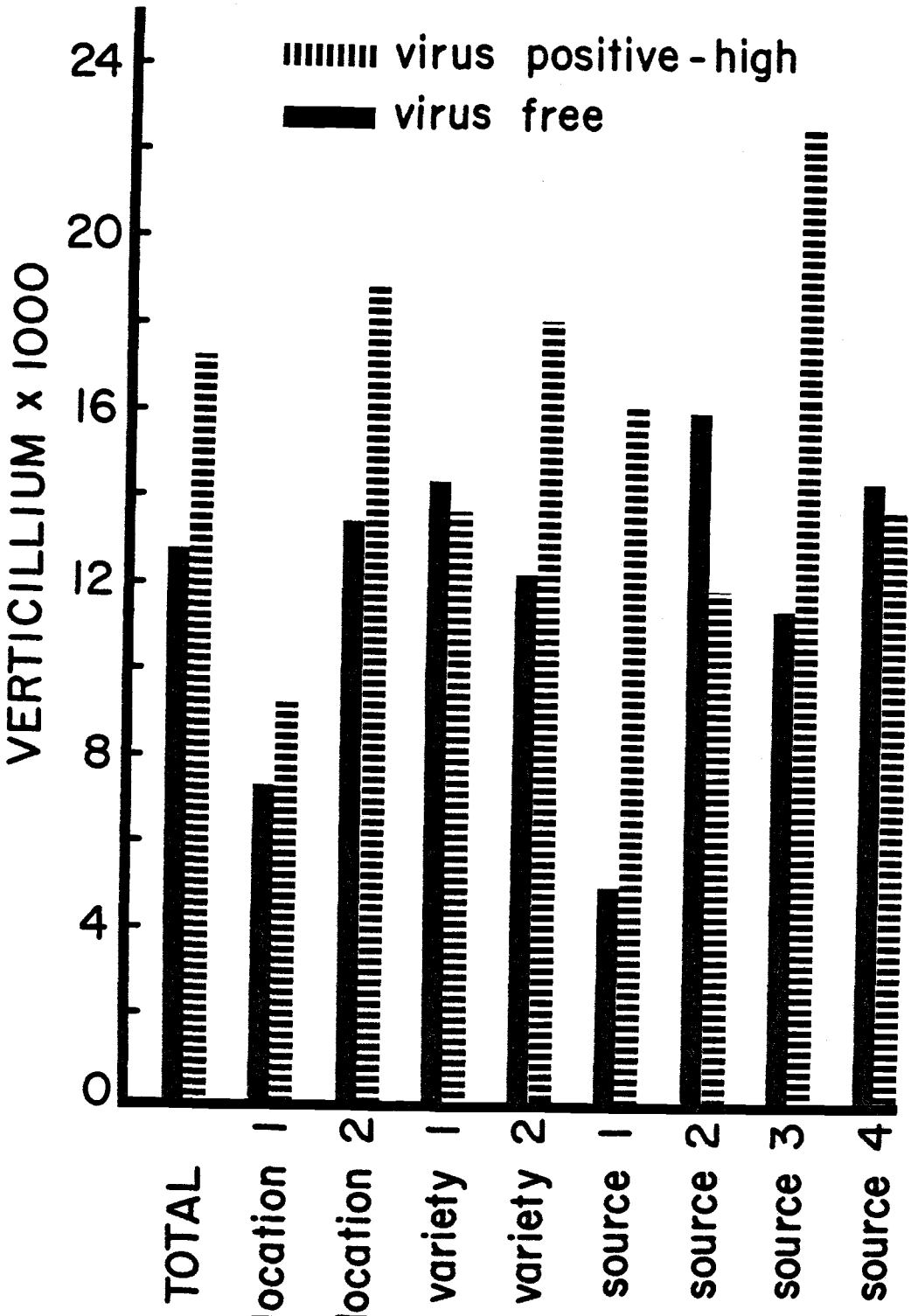

virus positive - high
virus free
VERTICILLIUM x 1000
0
4
8
12
16
20
24
TOTAL
location 1
location 2
variety 1
variety 2
source 1
source 2
source 3
source 4

Table 5. The effect of PVX infection on the mean ln severity of Verticillium dahliae infected plants across all locations, varieties and source.

	mean ln units/cm at stem base	2-tail random prob.
PVX absent	8.2473	.001
High level PVX present	8.8435	

smaller difference between the mean severity levels of V. dahliae in the virus-free and the virus-positive plants and to a high level of variability of V. dahliae levels within the population, the observed random probability of PVX exerting an effect on V. dahliae colonization is higher, (α = 0.113)(Table 6). Norgold Russets also showed a lower level of significant interaction between PVX and V. dahliae in previous tests.

When a similar P_{rb} analysis was done, first partitioning by location, with location 1 representing the control plots where coincidence and vascular colonization were low and location 2 representing a field with a high wilt potential, Verticillium colonization was

Table 6. The effect of PVX infection on the mean ln severity of Verticillium dahliae infected plants across both locations for 'Norgold Russets' and 'Russet Burbanks'.

	mean ln propagules/cm^2 at stem base	2-tailed random prob.
'Russet Burbanks' PVX absent	8.1946	.000
'Russet Burbanks' high level PVX present	8.9178	
'Norgold Russets' PVX absent	8.4430	.113
'Norgold Russets' high level PVX present	8.5512	

observed to be greater in the presence of PVX (Table 8) at both locations. The difference was most pronounced at location 2 with a probability level of 0.000 vs. a random probability of 0.175 for location 1. This indicates that most of the observed significance was due to a strong relationship between PVX and V. dahliae colonization in fields where early dying of potato vines was severe.

Upon partitioning the Russet Burbanks by seed lot it was observed that seed lots 1 and 3 show higher mean ln V. dahliae levels in the presence of PVX with random probability levels of 0.000, and 0.001 respectively. When mean ln Verticillium levels in PVX-high vs PVX-free plants are analyzed in seed source 2 the mean ln Verticillium level is higher in the virus-free plant but this relationship fails to show significance at the 90% level for virus-free vs. virus-high analysis of mean ln V. dahliae severity (Table 9).

The analyses described above were repeated using a virus-free vs. a virus present dichotomy; i.e., including in the analysis all PVX-infected plants rather than just those which showed a high virus content as would be expected from seed piece or early season infection. The random probability levels were somewhat higher but the relationship between PVX and V. dahliae severity was still evident.

A total of 49 independent probability evaluations were made. The ones discussed above are a sample of those done using the natural log of the severity ratings instead of actual units per cm. Analysis was done on the basis of natural logs because yield analysis done previously indicated that yield is a function of the interaction of incidence x ln severity of V. dahliae infection. Eighty-eight percent of the variation in yield between new and old locations can be

TABLE 7. The effect of PVX infection on the mean ln severity of Verticillium dahliae infected plants of both varieties at location one and location two.

		mean ln propagules/ cm at stem base	2-tailed random prob.
Location 1	PVX absent	7.3419	.175
Location 1	high level PVX present	7.8509	
Location 2	PVX absent	8.3755	.000
Location 2	high level PVX present	9.0452	

TABLE 8. The effect of PVX infection on the mean ln severity of Verticillium dahliae infected plants across both locations for three 'Russet Burbank' seed sources.

		mean ln propagules/ cm^2 at stem base	2-tailed random prob.
Seed source 1	PVX absent	7.0085	.000
Seed source 1	high level PVX present	8.7897	
Seed source 2	PVX absent	8.5683	.260
Seed source 2	high level PVX present	8.3039	
Seed source 3	PVX absent	8.3142	.001
Seed source 3	high level PVX present	9.3030	

explained by the interaction of incidence x ln severity of V. dahliae infection. When the analyses were repeated using untransformed data the random probability levels obtained varied but in general remained low. The patterns observed among the means upon partitioning remained the same (see Appendices v, vi, vii, viii, ix, and x).*

Figures 8 and 9 summarize the relationship found between PVX and V. dahliae mean severity of infected plants for virus-free vs. virus-positive plants and for virus free plants vs. plants with a high level of virus respectively. Note that whereas Figures 6 and 7 dealt with the level of V. dahliae infection averaged over all plants (whether or not they were infected with V. dahliae), Figures 8 and 9 reflect the average level of colonization found in plants infected with V. dahliae.

3. Effect of PVX on Incidence and Stem Colonization by Colletotrichum atramentarium

The mean number of propagules per cm at stem base when averaged for both Colletotrichum infected and C. atramentarium free plants is higher in the PVX free plants. Averages for virus infected and PVX free plants were 1826.5 and 2845.9, respectively. Two-tailed random probability is 0.0132. Partitioning by location, variety, seed source

*When comparing between analyses run with untransformed data and those using natural logs the following should be noted: 1) The mean ln severity is not equal to the ln of the mean severity and 2) Just because one mean severity is greater than another, the ln mean severities of the two groups need not necessarily follow the same relationship. For two of the 98 analyses it was seen that mean severity ('A') was greater than mean severity 'B' while mean ln severity 'A' was less than mean ln severity 'B'. The above two points are consequences of the non-linear nature of the transformation to natural logs and underscore the need for parallel analysis.

Figure 8. Mean level of colonization of Verticillium dahliae in infected plants for PVX-free versus PVX-positive plants.
Location 1 = new field
Location 2 = old field
Variety 1 = 'Norgold Russet' seed source 4
Variety 2 = 'Russet Burbank' seed sources 1, 2 and 3

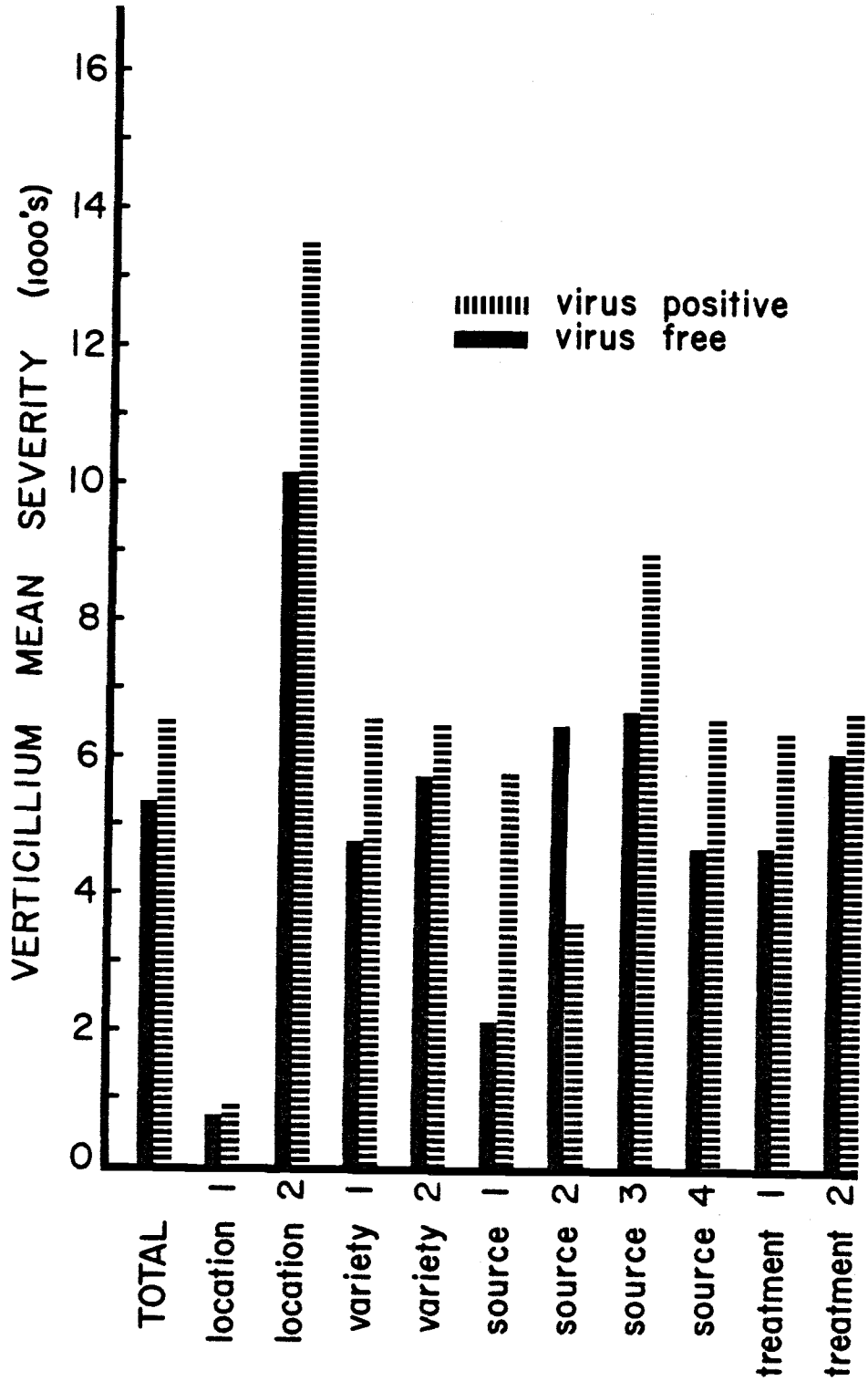
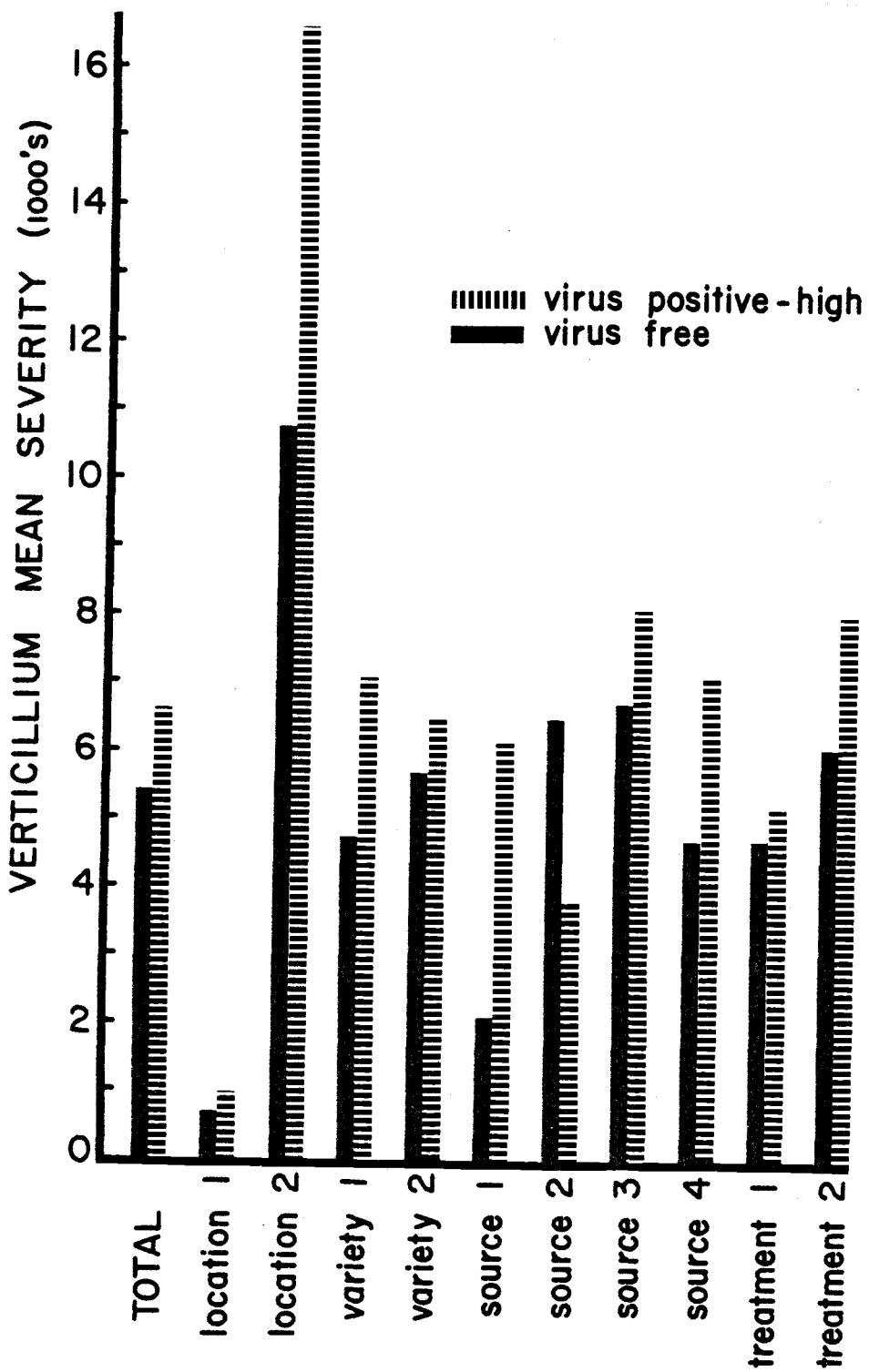

VERTICILLIUM MEAN SEVERITY (1000's)
0
2
4
6
8
10
12
14
16
virus positive
virus free
TOTAL
location 1
location 2
variety 1
variety 2
source 1
source 2
source 3
source 4
treatment 1
treatment 2

Figure 9. Mean level of colonization of Verticillium dahliae in infected plants for PVX-free plants versus plants with a high level of PVX.
Location 1 = new field
Location 2 = old field
Variety 1 = 'Norgold Russet' seed source 4
Variety 2 = 'Russet Burbank' seed sources 1, 2 and 3


VERTICILLIUM MEAN SEVERITY (1000's)
0
2
4
6
8
10
12
14
16
virus positive - high
virus free
TOTAL
location 1
location 2
variety 1
variety 2
source 1
source 2
source 3
source 4
treatment 1
treatment 2

and treatment, C. atramentarium severity X incidence value is found to be consistently higher in the virus-free plants (Figure 10).

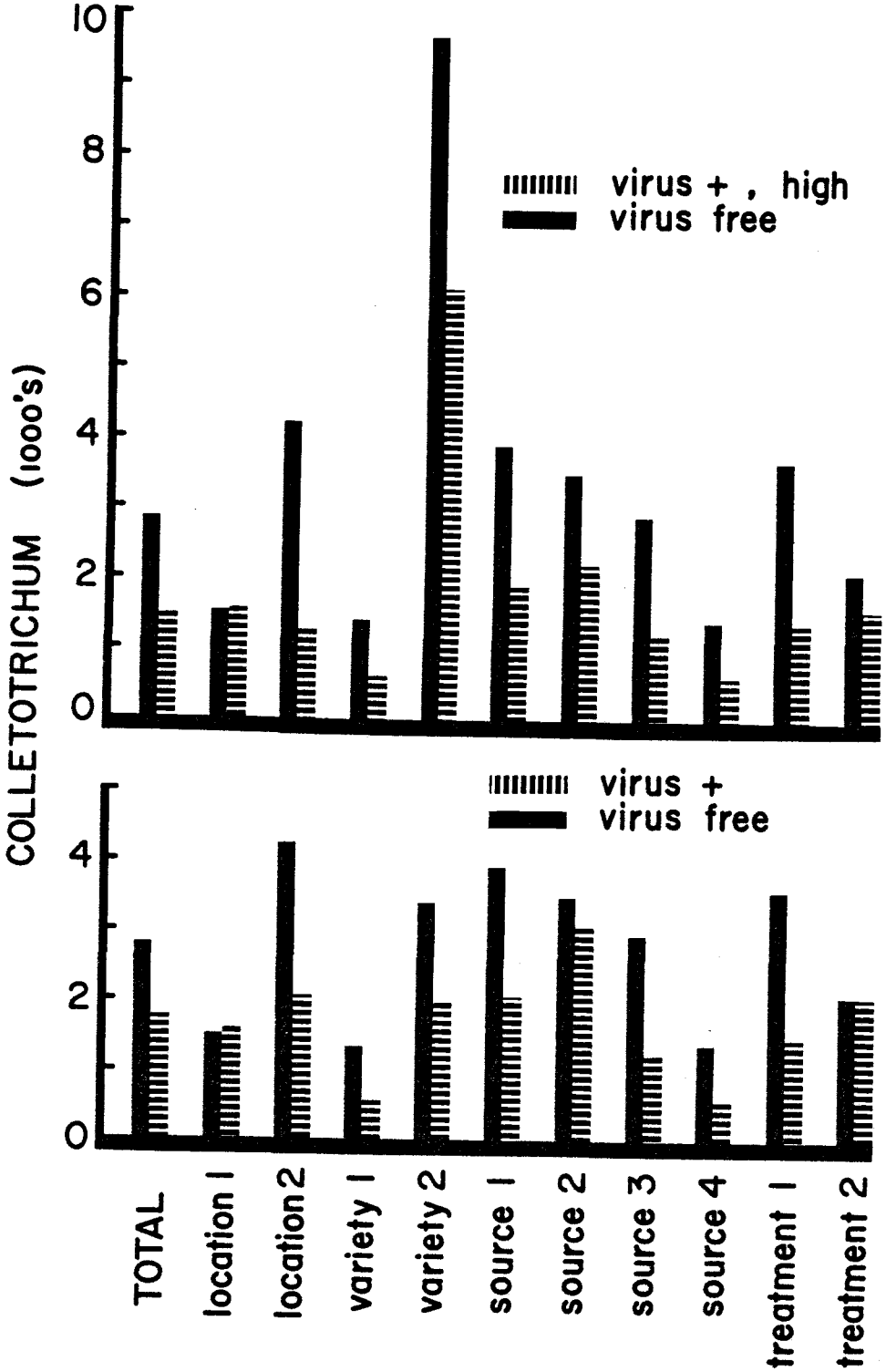
TABLE 9. T-test of the mean C. atramentarium value for virus positive and virus free plants.

	# cases	Mean coll. value	R_{pb}	T	2-tail prob.
Virus-positive	782	1826.51	-0.0663	-2.48	.0132
Virus-free	584	2845.88			

These results are not in keeping with the characterization of C. atramentarium as a saprophyte or weak pathogen which would lead to expectations of a higher level of C. atramentarium infection in the less vigorous virus infected plants. These results are, however, in keeping with observations by J. Davis that high C. atramentarium levels are associated with optimal plant nutrition and the adverse effect of PVX on nitrogen and phosphorous levels in Norgold Russets (27, 28, 123). Means for C. atramentarium infection obtained upon partitioning and their individual probability levels are given in Appendix vi.

X^2 analysis comparing observed versus expected incidence frequencies were run to determine if the strongly inverse relationship between PVX incidence and C. atramentarium incidence was due to an effect of PVX on C. atramentarium incidence. The X^2 value obtained for variety 'Russet Burbank' was 26.3 with a random probability of .01. No significant relationship was observed between PVX and C. atramentarium incidence in variety 'Norgold Russet' with an X^2 value of 1.5.

Figure 10. Mean Colletotrichum atramentarium propagules per cm^2 at stem base averaged over both Colletotrichum atramentarium infected and non-infected plants and shown for PVX positive versus PVX-free plants (lower graph) and for PVX-free plants versus plants showing a high level of PVX infection (upper graph).
Location 1 = new field
Location 2 = old field
Variety 1 = 'Norgold Russet' seed source 4
Variety 2 = 'Russet Burbank' seed sources 1, 2 and 3
Treatment 1 = noninoculated
Treatment 2 = inoculated with Verticillium dahliae


COLLETOTRICHUM (1000's)
10
8
6
4
2
0
virus + , high
virus free
4
2
0
virus +
virus free
TOTAL
location 1
location 2
variety 1
variety 2
source 1
source 2
source 3
source 4
treatment 1
treatment 2

When C. atramentarium colonization of infected plants is examined as a function of PVX (Tables 11, 12, 13, and 14) the highest C. atramentarium levels are consistently associated with virus freedom. This was true regardless of location, variety or seed source. Across both locations and all four seed sources, the random probability for virus-free versus high PVX present is .002 when the analysis is done on the basis of natural logs. Most of the significance is due to a strong relationship at location 2, the old field, in which early dying is pronounced. The relationship is strongest among the 'Russet Burbank' variety with a random probability of .000. The same pattern of the means is observed for the 'Norgold Russet' variety but the random probability is higher (.228).

Additional probability evaluations were conducted using different partitionings and a virus free vs. virus present dichotomy. Results are presented in Appendices xi, xii, xiii, xiv, xv and xvi. All analyses were repeated using non-transformed data. Random probability levels were in general somewhat higher than when the analyses were run using natural logs but the inverse relationship between PVX and C. atramentarium was still evident. This relationship is expressed graphically in Figure 11 where the level of C. atramentarium colonization of infected plants is greater in PVX-free plants at both locations and for all seed sources.

4. A Possible Relationship Between Verticillium dahliae and Colletotrichum atramentarium

In investigating the inverse relationship between PVX and C. atramentarium a series of X^2 tests were made of C. atramentarium vs.

TABLE 10. The effect of PVX infection on the mean ln severity of C. atramentarium infected plants across all locations, varieties and sources.

	mean ln propagules/ cm at stem base	2-tailed random prob.
PVX absent	8.2473	.002
high level PVX present	8.6907	

TABLE 11. The effect of PVX infection on the mean ln severity of C. atramentarium infected plants across all locations for Russet Burbanks and Norgold Russets.

		mean ln propagules/ cm at stem base	2-tailed random prob.
Russet Burbank	PVX absent	8.5444	.000
Russet Burbank	high level PVX present	7.7249	
Norgold Russet	PVX absent	7.6098	.228
Norgold Russet	high level PVX present	7.2630	

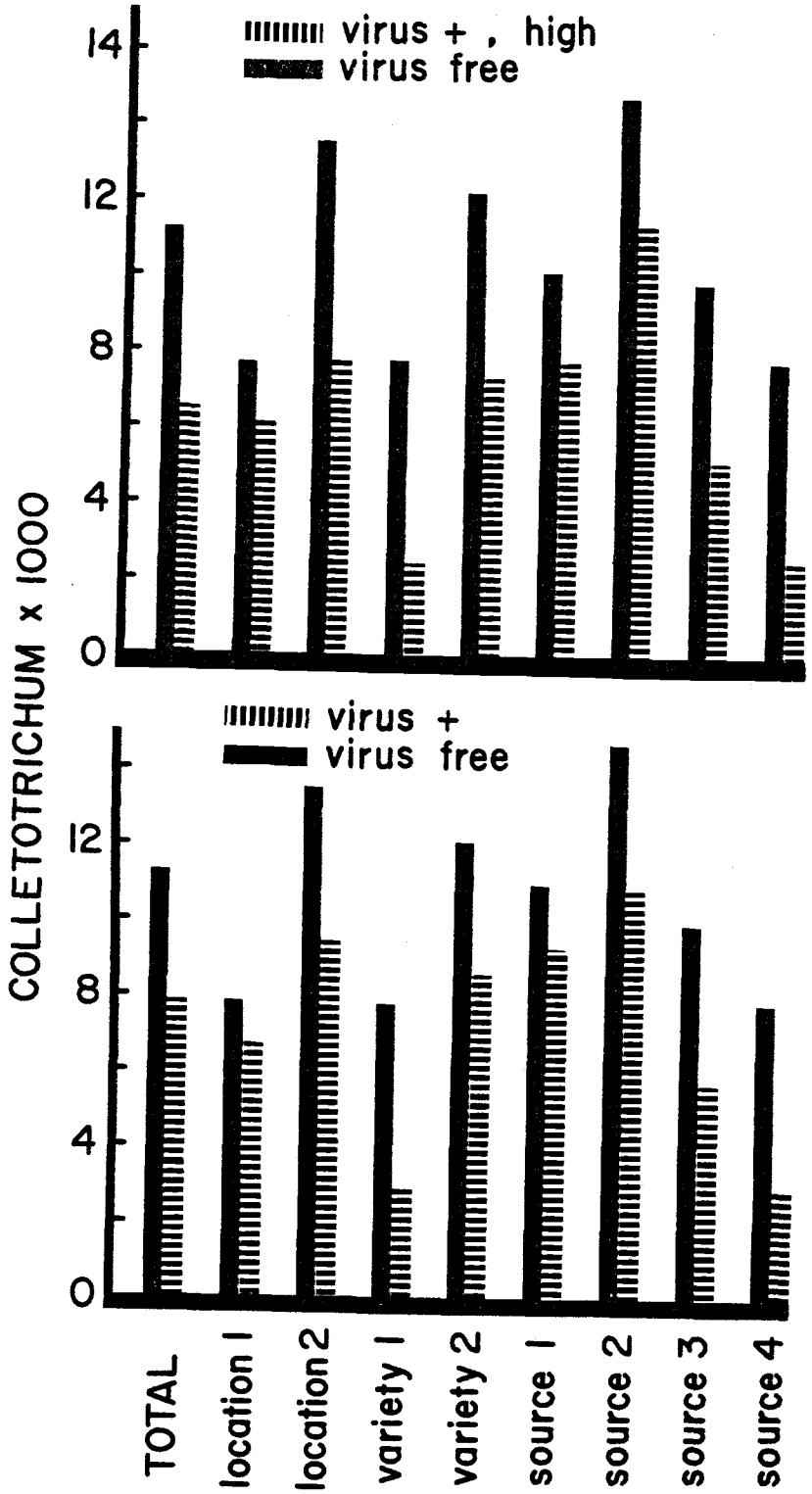
TABLE 12. The effect of PVX infection on the mean ln severity of C. atramentarium infected plants of both varieties at location one and location two.

		mean ln propagules/ cm at stem base	2-tailed random prob.
Location 1	PVX absent	7.5511	.319
Location 1	high level PVX present	7.4098	
Location 2	PVX absent	8.8768	.017
Location 2	high level PVX present	8.3142	

TABLE 13. The effect of PVX infection on the mean ln severity of C. atramentarium for three Russet Burbank seed sources.

		mean ln propagules/ cm at stem base	2-tailed random prob.
Seed source 1	PVX absent	8.1464	.125
Seed source 1	high level PVX present	7.6600	
Seed source 2	PVX absent	8.7330	.155
Seed source 2	high level PVX present	8.2633	
Seed source 3	PVX absent	8.6155	.001
Seed source 3	high level PVX present	7.4978	

Figure 11. Mean level of colonization of Colletotrichum atramentarium in infected plants for PVX-free plants versus PVX-positive plants (lower graph) and for PVX-free plants versus plants with a high level of PVX (upper graph).
Location 1 = new field
Location 2 = old field
Variety 1 = 'Norgold Russet' seed source 4
Variety 2 = 'Russet Burbank' seed sources 1, 2 and 3


virus + , high
virus free
virus +
virus free
COLLETOTRICHUM x 1000
0
4
8
12
14
TOTAL
location 1
location 2
variety 1
variety 2
source 1
source 2
source 3
source 4

V. dahliae incidence. Smaller than expected numbers of coincident C. atramentarium and V. dahliae infections and higher than expected numbers of sole infections by either organism were observed with the 'Russet Burbank' seed lots ($X^2 = 29.9$; $\alpha = 0.01$). Similar results are observed for both locations. These results are in disagreement with observations made by other researchers of a tendency for Colletotrichum to be associated with concurrent V. dahliae infection (47).*

C. Conclusions

The presence of PVX had no effect upon V. dahliae incidence, but PVX did exert a significant influence upon V. dahliae colonization. A marked increase in V. dahliae colonization was observed in plants coinfected with PVX. The effect of PVX on severity could be due to a general loss of vitality in the virus-infected plants as other studies have indicated that field resistance to V. dahliae is closely related to plant vigor (29). The effect PVX exerted on V. dahliae colonization was most pronounced in the field in which early dying was severe and appeared to be seed source dependent. The significance of the effect PVX exerted on V. dahliae lies in the fact that yield losses observed in fields suffering from early dying of potato vines are effectively explained by the level of V. dahliae present in the field (95).

An inverse relationship was observed for PVX and C. atramentarium. For both locations, treatments, varieties and all seed sources the

*The relationship between Verticillium and Colletotrichum cannot be explained on the basis of virus-free, Verticillium-low, plants having a thicker stem and thus more area for Colletotrichum to colonize as incidence should be independent of stem diameter.

incidence and severity of C. atramentarium was greater in the virus-free plants. This is an unexpected relationship but can perhaps be explained in part by realizing the deleterious effect PVX can have on plant nutrition.

Recent observations by J. Davis indicate that C. atramentarium levels may be higher in well fertilized fields (28). The strong relationship between PVX and C. atramentarium observed in this study should be further investigated in light of recent reports which indicate that C. atramentarium possesses a higher level of pathogenicity than previously acknowledged (124, 127).

Coincident infection of C. atramentarium and V. dahliae was observed to be lower in the 1200 Russet Burbank plants tested than expected with a random probability of 0.01. This is not in keeping with published reports and should also be investigated further.

In summary, PVX appears to play a significant role, possibly via a vigor mechanism, in the disease situation present in fields suffering from early dying of potato vines. The strong relationship between PVX and the severity of V. dahliae colonization, a primary yield determinant in these fields, suggests that planting virus-free seed would be a useful partial cultural control practice in areas where early dying of potato vines is prevalent.

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APPENDICES

Appendix i

Selected list of chemicals and equipment used.

Alkaline phosphatase, type VII
No. P-4502, Sigma Chemical Co.

Beckman Model 25 spectrophotometer, 80 ul 10 mm path length flow cell no. 886878, Beckman Instruments Co.

Bovine serum albumin, crystallized and lyophilized
No. A7638, Sigma Chemical Co.

Diethanolamine, Cat. 1598, Eastman Organic Chemicals

Egg albumin
No. A-5253, Sigma Chemical Co.

Glow Box Model GB 11-8
Instruments for Research & Industry

Glutaraldehyde - electron microscope grade
No. G-5882, Sigma Chemical Co.

Microelisa substrate plates, flat-bottom
No. 1-233-29, Dynatech Laboratories, Inc.

p-nitrophenyl phosphate, disodium
No. 104-40T, Sigma Chemical Co.

Polyvinylpyrrolidone, MW 10,000 or MW 40,000
No. PVP-10 or PVP-40, Sigma Chemical Co.

Silicone coating
No. Sigmacote SI-1, Sigma Chemical Co.

Tween 20 - (Polyoxyethylene sorbitan monolaurate)
No. P-1379, Sigma Chemical Co.

Appendix ii

Buffers used:

Coating buffer pH 9.6

Na_2CO_3	1.59 g
$NaHCO_3$	2.93 g
NaN_3	0.2 g
H_2O	1 l.

PBS - Tween washing solution pH 7.4

NaCl	8.0 g
KH_2PO_4	0.2 g
Na_2HPO_4 - 12 H_2O	2.9 g
KCl	0.2 g
NaN_3	0.2 g
Tween-20	0.5 ml
H_2O	1 l.

Virus and conjugate buffer pH 7.4

PBS-Tween	1 l.
(Polyvinylpyrrolidone MW 40 or MW 10)	20 g)
(Ovalbumin	2g)

Substrate buffer pH 9.8

diethanolamine	97 ml
NaN_3	0.2 g
H_2O	903 ml

Appendix iii. Comparison between ELISA and Gomphrena globosa bioassay diagnosis of PVX.

Treatment	Repl.	Assay used	Plant identification letter a b c d e f g h i j k l m n o p q r s t u v w x y z a' b' c' d'
Control	I	G. globosa	- -
		ELISA	- - - - - - + + - - - - + + - - - - - - - - - - - - - -
	II	G. globosa	+ + - + + - - + - - - + - - + + - - - + + - - - + - - - - -
		ELISA	- -
	III	G. globosa	- - - - - - - - + - - - - - - - - + - + + - + + - - - - + +
		ELISA	- - - - - - - - + - - - - - - - - + + + - + + - - - - + +
	IV	G. globosa	- - + - - - - + + - - - - - - - - - - - - - - - - - - + + + +
		ELISA	- - + - + + - -
mild PVX inoc. at cutting time	I	G. globosa	+ + + + + - + - + + + + + + - + - + - + + 0 - - - - + - - -
		ELISA	- - - - - - - - - - + - + - - - - - - - - - 0 - - - - - - - -
	II	G. globosa	- + - + + + - - - - - - - + + - - - - + - - - + + + + - - -
		ELISA	- + + - - + - + + + + + + + - - - + + - - - - - - - - - -
	III	G. globosa	- - - + - + - + + - - -
		ELISA	- + - - - -

- = PVX diagnosed as not present
+ = PVX diagnosed as present
0 = sufficient data not available

Appendix iii (continued)

Treatment	Repl.	Assay used	Plant identification letter																													
			a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	s	t	u	v	w	x	y	z	a'	b'	c'	d'
	IV	G. globosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		ELISA	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
mild PVX inoc. July 31st	I	G. globosa	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+
		ELISA	+	+	+	-	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+	+	+	+
	II	G. globosa	+	+	-	+	+	-	+	-	-	-	-	-	+	+	+	+	+	+	-	+	+	+	0	+	+	+	+	+	-	+
		ELISA	-	+	-	-	-	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	0	+	+	+	+	-	-	+
	III	G. globosa	+	+	+	+	+	+	+	+	+	+	0	+	+	+	+	+	+	0	+	+	+	+	+	+	+	+	+	+	+	+
		ELISA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	-	+	+	+	+	+	-
	IV	G. globosa	-	+	-	+	+	-	-	+	-	+	+	-	-	+	+	+	+	-	-	+	-	+	-	-	-	+	+	+	+	+
		ELISA	-	+	-	-	-	-	-	+	+	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
severe PVX inoc. July 31st	I	G. globosa	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		ELISA	-	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	-	+	+
	II	G. globosa	+	+	+	-	-	+	+	+	+	+	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
		ELISA	-	+	+	-	-	-	-	-	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+

- = PVX diagnosed as not present
+ = PVX diagnosed as present
0 = sufficient data not available

Appendix iii (continued)

Treatment	Repl.	Assay used	Plant identification letter																											
			a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	s	t	u	v	w	x	y	z	a'	b'	c'	d'
	III	*G. globosa*	+	+	-	+	+	-	-	+	+	+	-	-	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+
		ELISA	+	+	+	+	-	-	-	+	+	-	-	-	+	+	-	+	-	+	-	-	-	+	+	-	-	+	+	+	+	-
	IV	*G. globosa*	+	-	-	-	-	-	+	-	-	-	+	+	+	-	-	+	+	+	+	+	-	-	+	+	+	+	0	0	0	0
		ELISA	+	-	-	-	+	+	-	-	-	-	-	+	+	+	-	+	-	+	+	+	-	-	-	+	+	+	0	0	0	0

- = PVX diagnosed as not present
+ = PVX diagnosed as present
0 = sufficient data not available

Appendix iv. Visual Evaluation of ELISA Results

Preliminary studies on 564 tuber and leaf samples showed that a high level of accuracy was obtainable using visual inspection of completed ELISA plates instead of spectrophotometric analysis for qualitative work. This was further investigated because visual evaluation used in conjunction with lyophylized reagents (108) represents a significant simplification of the ELISA procedure.

An additional 1535 potato stem samples were tested using ELISA as described previously and evaluated spectrophotometrically and visually. Visual ratings ranged from 0 (colorless) to 6 (very strong yellow color). Spectrophotometric measurements of duplicate wells were averaged and considered positive if OD_{405} was at least two times the highest value given by any of the healthy controls on the same plate. Results were analyzed to determine the range and mean of OD's corresponding to the different visual ratings, their skew, kurtosis and confidence interval. The final predictive value of visual ratings for PVX presence or freedom was also evaluated.

Of the 1535 samples 615 were rated visually as 0, 240 as 1, 318 as 2, and 362 as 3, 4, 5 or 6. The mean OD_{405} increased with increasing visual rating. Ranges of OD_{405} obtained for each individual rating were extreme, for example, visual rating 5 had a mean OD of .568 but a range of OD's from .247 to 1.14. Visual rating 0 ranged in OD all the way up to 1431, effectively overlapping the OD ranges obtained for the five other ratings. As evaluated on the basis of range alone, visual ratings appear to be a very poor indicator of spectrophotometric evaluation. However, these large ranges are accompanied by

Table i. Parameters associated with the different visual ratings used in evaluating ELISA plates.

Visual Rating	# Cases	Mean OD_{405}	Range OD_{405}	Skew	Kurtosis	Var.	95% CI
0	615	.041	0-.431	3.97	23.70	.002	.037
1	240	.134	.028-1.32	7.95	88.30	.010	.122
2	318	.222	.026-.868	2.24	14.81	.006	.214
3	66	.257	.002-.500	0.32	-.311	.011	.232
4	250	.447	.017-2.59	3.90	20.11	.090	.410
5	22	.568	.247-1.14	1.40	4.39	.032	.489
6	24	.666	.357-.911	-.05	-.05	.019	.603

Table ii. Visual = 0 as a predictor for final virus free evaluation.

	Absolute freq.	Relative freq.	Adjusted freq.
Virus free	596	96.9	97.1
Low virus level	11	1.8	1.8
High virus level	7	1.1	1.1
(missing data)	1	0.2	
	615		

Table iii. Visual = 0 as a predictor for final virus positive evaluation.

	Absolute freq.	Relative freq.	Adjusted freq.
Virus positive	824	89.6	91.5
Virus free	77	8.4	8.5
(missing data)	19	0.2	
	920		

Table iv. Visual = 0 or 1 as a predictor for final virus positive evaluation.

	Absolute freq.	Relative freq.
Virus positive	676	99.4
Virus free	4	0.6
	680	

Table v. Visual = 0 or 1 as a predictor for final virus free evaluation.

	Absolute freq.	Relative freq.
Virus free	693	81.1
Low virus level	143	16.7
High virus level	18	2.0
(missing data)	1	
	855	

very high kurtosis ratings especially for the ratings 0, 1 and 2 which are the critical borderline ratings for qualitative evaluation. A measure of kurtosis is obtained from the second and fourth moments (with a moment around the arithmetic mean defined as $\frac{\Sigma(x - \bar{x})^r}{N}$ and kurtosis $g_2 = \frac{m_4}{m_2^2} - 3$. For a normal distribution $g_2 = 0$.)

The kurtosis ratings obtained for ratings 0, 1 and 2 are 23.7, 88.3 and 14.8 respectively. This indicates a high degree of "peakedness" with regard to their frequency distribution when plotted as a function of OD_{405}. Values of a related measure, skewness, obtained from the second and third moments, are given for the visual ratings in Table i, Appendix iv.

Looking at Table ii, Appendix iv, it is seen that virus freedom can be predicted with an accuracy of 97% using visual = 0 as a predictor. If visual $\neq$ 0 is used as a predictor for final virus positive evaluation, an accuracy level of 91.5% is obtained (Table iii). This indicates that by regarding all samples yielding a visual rating of 0 as virus negative it is possible to correctly identify 97.1% of the material correctly. Three percent of the material will actually be PVX-infected but will have escaped detection visually. Concommitantly, one will be accepting all samples with a visual rating of 2, 3, 4, 5, or 6 as virus infected. Only 91.5% of these samples will in actuality be virus-infected.

If visual $\neq$ 0 or 1 is used as a predictor for a final virus positive evaluation, 99.4% of the samples can be correctly identified, with 0.6% of the samples identified as virus positive actually being

virus free as determined by spectrophotometric analysis. The error of the opposite is correspondingly greater with 18.7% of the samples being identified as virus free which are actually virus positive (Tables iv and v).

In general, visual ratings though unsuited for quantitative work are capable of predicting virus presence (as determined spectrophotometrically) with a reasonable accuracy. These results indicate a possible role for visual evaluation of ELISA in certification or extension situations. For example in the rouging of infected plants where one is willing to risk the removal of a few healthy plants.

Appendix v. Mean V. dahliae level averaged over all plants and expressed as propagules per cm at the stem base for PVX-infected and PVX-free plants.

Partition	Virus level	Mean V. dahliae	2 tail rand. P
Total	absent	5446	.119
	present	6529	
Location 1	absent	766	.751
	present	874	
Location 2	absent	10174	.008
	present	13549	
Variety 1	absent	4750	.270
	present	6602	
Variety 2	absent	5711	.302
	present	6519	
Seed source 1	absent	2078	.000
	present	5785	
Seed source 2	absent	6523	.019
	present	3591	
Seed source 3	absent	6694	.105
	present	9034	
Seed source 4	absent	4750	.270
	present	6602	
Treatment 1	absent	4764	.066
	present	6403	
Treatment 2	absent	6076	.578
	present	6666	

Appendix vi. Mean V. dahliae level averaged over all plants and expressed as propagules per cm at the stem base for PVX-infected plants showing a high level of infection and PVX-free plants.

Partition	Virus level	Mean V. dahliae	2 tail rand. P
Total	absent	5446	.209
	present	6531	
Location 1	absent	766	.689
	present	917	
Location 2	absent	10174	.000
	present	16635	
Variety 1	absent	4750	.217
	present	7109	
Variety 2	absent	5711	.461
	present	6430	
Seed source 1	absent	2078	.008
	present	6126	
Seed source 2	absent	6523	.057
	present	3800	
Seed source 3	absent	6694	.413
	present	8070	
Seed source 4	absent	4750	.217
	present	7109	
Treatment 1	absent	4764	.714
	present	5127	
Treatment 2	absent	6076	.171
	present	8023	

Appendix vii. Mean *V. dahliae* severity averaged over *V. dahliae* infected plants expressed as propagules per cm at the stem base for PVX-infected plants and PVX-free plants.

Partition	Virus level	Mean V. dahliae	2 tail rand. P
Total	absent	12780	.009
	present	16155	
Location 1	absent	7255	.304
	present	9281	
Location 2	absent	13562	.010
	present	17172	
Variety 1	absent	14507	.420
	present	15232	
Variety 2	absent	12315	.006
	present	16281	
Seed source 1	absent	5063	.000
	present	15076	
Seed source 2	absent	16062	.018
	present	10057	
Seed source 3	absent	11566	.001
	present	20344	
Seed source 4	absent	14507	.420
	present	15232	

Appendix viii. Mean V. dahliae severity averaged over V. dahliae infected plants expressed as propagules per cm at the stem base for PVX-infected plants showing a high level of virus infection and for virus-free plants.

Partition	Virus level	Mean V. dahliae	2 tail rand. P
Total	absent	12780	.005
	present	17293	
Location 1	absent	7255	.406
	present	9220	
Location 2	absent	13562	.003
	present	18934	
Variety 1	absent	14507	.422
	present	13742	
Variety 2	absent	12315	.001
	present	18196	
Seed source 1	absent	5063	.002
	present	16163	
Seed source 2	absent	16062	.129
	present	11950	
Seed source 3	absent	11566	.000
	present	22592	
Seed source 4	absent	14507	.422
	present	13742	

Appendix ix. Mean natural log V. dahliae severity averaged over V. dahliae infected plants expressed as propagules per cm at the stem base for PVX-infected plants and PVX-free plants.

Partition	Virus level	Mean V. dahliae	2 tail rand. P
Total	absent	8.2473	.002
	present	8.6907	
Location 1	absent	7.3419	.251
	present	7.6615	
Location 2	absent	8.3755	.002
	present	8.8429	
Variety 1	absent	8.4430	.356
	present	8.5904	
Variety 2	absent	8.1946	.001
	present	8.7044	
Seed source 1	absent	7.0085	.000
	present	8.6795	
Seed source 2	absent	8.5683	.018
	present	7.8981	
Seed source 3	absent	8.3142	.001
	present	9.1072	
Seed source 4	absent	8.4430	.356
	present	8.5904	

Appendix x. Mean natural log *V. dahliae* severity averaged over *V. dahliae* infected plants expressed as propagules per cm at the stem base for PVX-infected plants showing a high level of virus and PVX-free plants.

Partition	Virus level	Mean V. dahliae	2 tail rand. P
Total	absent	8.2473	.001
	present	8.8435	
Location 1	absent	7.3419	.175
	present	7.8509	
Location 2	absent	8.3755	.000
	present	9.0452	
Variety 1	absent	8.4430	.113
	present	8.5512	
Variety 2	absent	8.1946	.000
	present	8.9178	
Seed source 1	absent	7.0085	.000
	present	8.7897	
Seed source 2	absent	8.5683	.260
	present	8.3039	
Seed source 3	absent	8.3142	.001
	present	9.3030	
Seed source 4	absent	8.4430	.113
	present	8.5512	

Appendix xi. Mean C. atramentarium level averaged over all plants and expressed as propagules per cm at the stem base for PVX-infected and PVX-free plants.

Partition	Virus level	Mean C. atramentarium	2 tail rand. P
Total	absent	2845	.022
	present	1826	
Location 1	absent	1523	.880
	present	1596	
Location 2	absent	4242	.005
	present	2136	
Variety 1	absent	1426	.230
	present	647	
Variety 2	absent	3404	.008
	present	1981	
Seed source 1	absent	3936	.179
	present	2051	
Seed source 2	absent	3483	.724
	present	3131	
Seed source 3	absent	2936	.013
	present	1217	
Seed source 4	absent	1426	.230
	present	647	
Treatment 1	absent	3670	.002
	present	1512	
Treatment 2	absent	2132	.985
	present	2142	

Appendix xii. Mean C. atramentarium level averaged over all plants and expressed as propagules per cm at the stem base for PVX-infected plants showing a high level of infection and PVX-free plants.

Partition	Virus level	Mean C. atramentarium	2 tail rand. P
Total	absent	2845	.006
	present	1508	
Location 1	absent	1523	.911
	present	1548	
Location 2	absent	4242	.000
	present	1362	
Variety 1	absent	1426	.249
	present	671	
Variety 2	absent	9751	.003
	present	6099	
Seed source 1	absent	3936	.176
	present	1882	
Seed source 2	absent	3483	.273
	present	2268	
Seed source 3	absent	2936	.015
	present	1169	
Seed source 4	absent	1426	.249
	present	671	
Treatment 1	absent	3670	.002
	present	1407	
Treatment 2	absent	2132	.416
	present	1610	

Appendix xiii. Mean C. atramentarium severity averaged over C. atramentarium infected plants expressed as propagules per cm at the stem base for PVX-infected and PVX-free plants.

Partition	Virus level	Mean C. atramentarium	2 tail rand. P
Total	absent	11272	.001
	present	7842	
Location 1	absent	7794	.298
	present	6743	
Location 2	absent	13540	.031
	present	9362	
Variety 1	absent	7779	.113
	present	2917	
Variety 2	absent	12168	.011
	present	8454	
Seed source 1	absent	10883	.309
	present	9272	
Seed source 2	absent	14683	.118
	present	10882	
Seed source 3	absent	9940	.011
	present	5620	
Seed source 4	absent	7779	.113
	present	2917	

Appendix xiv. Mean C. atramentarium severity averaged over C. atramentarium infected plants expressed as propagules per cm at the stem base for PVX-infected plants showing a high level of virus infection and PVX-free plants.

Partition	Virus level	Mean C. atramentarium	2 tail rand. P
Total	absent	11272	.005
	present	6533	
Location 1	absent	7794	.220
	present	6099	
Location 2	absent	13539	.041
	present	7761	
Variety 1	absent	7779	.118
	present	2572	
Variety 2	absent	12168	.008
	present	7347	
Seed source 1	absent	10883	.225
	present	7819	
Seed source 2	absent	14683	.218
	present	11298	
Seed source 3	absent	9940	.020
	present	5066	
Seed source 4	absent	7779	.118
	present	2572	

Appendix xv. Mean natural log C. atramentarium severity averaged over C. atramentarium infected plants expressed as propagules per cm^2 at the stem base for PVX-infected plants and PVX-free plants.

Partition	Virus level	Mean C. atramentarium	2 tail rand. P
Total	absent	8.3537	.021
	present	8.0016	
Location 1	absent	7.5511	.497
	present	7.5492	
Location 2	absent	8.8768	.078
	present	8.6266	
Variety 1	absent	7.6098	.249
	present	7.3228	
Variety 2	absent	8.5444	.007
	present	8.0859	
Seed source 1	absent	8.1464	.341
	present	8.2824	
Seed source 2	absent	8.7330	.066
	present	8.2175	
Seed source 3	absent	8.6155	.004
	present	7.7696	
Seed source 4	absent	7.6098	.249
	present	7.3228	

Appendix xvi. Mean natural log C. atramentarium severity averaged over C. atramentarium infected plants expressed as propagules per cm^2 at the stem base for PVX-infected plants showing a high virus level and PVX-free plants.

Partition	Virus level	Mean C. atramentarium	2 tail rand. P
Total	absent	8.3537	.000
	present	7.6462	
Location 1	absent	7.5511	.319
	present	7.4098	
Location 2	absent	8.8768	.017
	present	8.3142	
Variety 1	absent	7.6098	.228
	present	7.2630	
Variety 2	absent	8.5444	.000
	present	7.7249	
Seed source 1	absent	8.1464	.125
	present	7.6600	
Seed source 2	absent	8.7330	.155
	present	8.2633	
Seed source 3	absent	8.6155	.001
	present	7.4978	
Seed source 4	absent	7.6098	.228
	present	7.2630	

Appendix xvii. Selected X^2 tests. P = random probability.

A. TEST OF V. DAHLIAE INCIDENCE VS. VIRUS RATING (0 = PVX absent; 1 = PVX present at a low level; 2 = PVX present at a high level)

Verticillium	PVX	Observed	Expected
absent	0	353	357
absent	1	247	252
absent	2	248	238
present	0	234	230
present	1	168	163
present	2	144	154

X^2 = 1.4
P = 99.9

B. TEST OF C. ATRAMENTARIUM INCIDENCE VS. VIRUS RATING

Colletotrichum	PVX	Observed	Expected
absent	0	437	444
absent	1	305	302
absent	2	298	292
present	0	147	140
present	1	93	95
present	2	88	92

X^2 = 1.5
P = 99.9

C. TEST OF C. ATRAMENTARIUM INCIDENCE VS. VIRUS RATING FOR RUSSET BURBANKS

Colletotrichum	PVX	Observed	Expected
absent	0	302	314
absent	1	277	208
absent	2	253	244
present	0	117	105
present	1	88	91
present	2	73	82

X^2 = 26.3
P = .01

Appendix xvii (continued)

D. COINCIDENCE OF V. DAHLIAE AND C. ATRAMENTARIUM

Verticillium	Colletotrichum	Observed	Expected
absent	absent	604	634
absent	present	241	204
present	absent	440	397
present	present	90	128

$X^2 = 23.9$
$P = .01$

E. COINCIDENCE OF V. DAHLIAE AND C. ATRAMENTARIUM IN NORGOLD RUSSETS

Verticillium	Colletotrichum	Observed	Expected
absent	absent	134	133
absent	present	33	34
present	absent	68	69
present	present	18	17

$X^2 = .04$
$P = .99$

F. COINCIDENCE OF V. DAHLIAE AND C. ATRAMENTARIUM IN RUSSET BURBANKS

Verticillium	Colletotrichum	Observed	Expected
absent	absent	470	509
absent	present	208	169
present	absent	372	333
present	present	72	111

$X^2 = 29.9$
$P = .01$