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THE IMPACT OF POTATO VIRUS Y
ON THE SEVERITY OF POTATO EARLY DYING

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ANA MARIA DA GRAÇA MONDJANA

A thesis submitted in partial fulfillment of the
requirements for the degree of

MASTERS OF SCIENCE
(PLANT PATHOLOGY)

at the
UNIVERSITY OF WISCONSIN-MADISON

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APPROVED BY:


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Under the supervision of Associate Professor Douglas I. Rouse

ABSTRACT

The effect of Verticillium dahliae on the severity of Potato Early Dying (PED) and on the potato yield in the presence of Potato Virus Y (PVY) was studied in two potato cultivars, Russet Norkotah and Russet Burbank. Field experiments were conducted at the Hancock Experiment Station during the summers of 1991 and 1992 in four row plots replicated five times per treatment in a randomized block design (RBD). PVY was mechanically inoculated on the foliage both years. Infected seed tubers harvested from infected plants in 1991 were used in additional treatments in 1992. Verticillium dahliae grown on rye grain was spread over the soil by hand and incorporated to a depth of 15 cm with a rototiller. Foliar infection by PVY resulted in a yield reduction of about 30% and 24% in Russet Norkotah and Russet Burbank respectively in 1991. In 1992, the effect of foliar inoculation treatment was not significant, either in viral symptom expression or on potato yield. This may have been due

to environmental conditions including frost damage following inoculation. Plants grown from PVY-infected tubers were found to have very good viral symptoms and to have reduced yield of about 50%. In 1991, V. dahliae alone did not show any effect on the development of PED. However, in 1992 the two levels of the fungus gave good symptom expression of PED and yield losses were 38% and 18% for Russet Norkotah and Russet Burbank respectively. PED severity and yield losses were greater when both diseases were present. The effects of combined diseases on the severity of PED were synergistic and on yield were additive.

In 1992 a field experiment was conducted to determine the effect of time of PVY infection on subsequent infection of the progeny tubers. The experiment was established at the Hancock Experimental Station using four row plots replicated four times per treatment in a RBD. Russet Norkotah and Russet Burbank potato plants were mechanically inoculated with PVY at three or four different dates, respectively. The results showed that earlier infection resulted in a higher number of infected progeny tubers.

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To my children, Joel and Nadia,
my sister Isabel,
my husband
my mother
and to the memory of my father
with love and gratitude.

TABLE OF CONTENTS

ABSTRACT.....	i
ACKNOWLEDGEMENTS.....	iii
TABLE OF CONTENTS.....	v
LIST OF TABLES.....	vi
LIST OF FIGURES.....	vii
INTRODUCTION.....	1
References cited.....	11
CHAPTER 1: Interaction between <u>Verticillium</u> <u>dahliae</u> and Potato Virus Y in potato	
Introduction.....	15
Materials and Methods.....	16
Results.....	27
Discussion.....	45
References cited.....	50
CHAPTER 2: Effect of timing of potato plant infection by PVY on tuber infection	
Introduction.....	53
Materials and Methods.....	54
Results.....	56
Discussion.....	58
References cited.....	59
CONCLUSIONS.....	60
References cited.....	63
APPENDIX I. 1992 greenhouse experiment.....	64
Introduction	64
Materials and Methods.....	65
Results and Discussion.....	68
References cited.....	75
APPENDIX II. Soil assay results.....	77
Tuber assay results.....	78
APPENDIX III. PVY characterization.....	79

LIST OF TABLES

Table		Page
CHAPTER ONE		
1.	Main cultural practices applied in 1991 and 1992.....	19
2.	Effect of PVY tuber infection on vascular colonization by <u>Verticillium dahliae</u> for Russet Norkotah and Russet Burbank.....	34
3.	Effect of the fungus on the virus content on the foliage of Russet Norkotah and Russet Burbank.....	35
4.	Green leaf area measurements on Russet Norkotah and Russet Burbank potato plants...	39
5.	Effect of PVY and <u>V. dahliae</u> on the specific gravity.....	46

LIST OF FIGURES

Figure		Page
	INTRODUCTION	
1.	PVY symptoms on potato plant.....	4
2.	PED symptoms on potato plant.....	7
	CHAPTER ONE	
3.	Microsclerotia colonies of <u>V. dahliae</u>	23
4.	PVY severity rating on the foliage of Russet Norkotah and Russet Burbank potato plants infected by PVY alone.....	28
5.	PED severity rating on Russet Norkotah and Russet Burbank potato plants infected by <u>V. dahliae</u> alone.....	30
6.	Effect of <u>V. dahliae</u> on the severity of PED in the presence of PVY for Russet Norkotah and Russet Burbank potato plants.....	32
7.	Percentage of progeny tubers infected by PVY in 1991 and 1992.....	36
8.	Effect of PVY and <u>V. dahliae</u> in the light interception on Russet Norkotah and Russet Burbank cultivars.....	38
9.	Effect of PVY alone on total potato yield for Russet Norkotah and Russet Burbank cultivars.	40
10.	Effect of <u>V. dahliae</u> alone on total potato yield for Russet Norkotah and Russet Burbank potato plants.....	42
11.	Effect of <u>V. dahliae</u> on total potato yield in the presence of PVY for Russet Norkotah and Russet Burbank potato plants.....	43

LIST OF FIGURES (continued)

12. Effect of V. dahliae on yield of A's tubers
in the presence of PVY for Russet Norkotah
and Russet Burbank cultivars..... 44

CHAPTER TWO

1. Effect of timing of inoculation by PVY on the
infection of progeny tubers..... 57

INTRODUCTION

The present study was part of a two-year research program on the interaction of Verticillium dahliae (Vd) with Potato Virus Y (PVY) on the severity of Potato Early Dying (PED) and potato yield.

PED and PVY are among the most important disease problems affecting the potato (Solanum tuberosum L.) crop in North America, as was revealed in a survey of potato growers (39). According to the respondents, PED was considered the most important potato disease problem in North America. Severity of PED depends on a variety of factors such as region, cultivar and environmental conditions (33). It is reported that PED may cause losses of 30% to 50% in many potato growing regions of the United States (33). Results from the survey showed that PVY was the 7th most important disease of potatoes overall and third most important disease of seed potatoes (39). The effect of PVY on potato yield is also highly variable (8). PVY reportedly may cause yield losses from 10% to 80% depending on the virus strain, potato cultivar and time of infection (8, 14).

POTATO VIRUS Y (PVY). PVY belongs to the potyvirus group. Members of the potyvirus group have filamentous particles, are transmitted by aphids, induce pinwheel

inclusions in the cytoplasm, and are serologically distinguishable from morphologically similar viruses (15). Many species, mostly solanaceae, are hosts of PVY.

PVY particles are flexuous filaments, measuring 730 nm in length and 11 nm in diameter. The particle contains a single stranded positive sense RNA genome, MWt about 3.1×10^6 (42).

Three groups of PVY strains are known to affect potatoes, namely Potato Virus Y⁰ (common strain), Potato Virus Y^N (Tobacco veinal necrosis strain) and Potato Virus Y^C (Stipple streak strain) (8, 17). According to Cockerham (4) the Y⁰ and Y^N strains differ from Y^C in their ability to overcome the dominant hypersensitivity gene Nc present in many potato cultivars. Jones et al. (17) stated that the strain Y^N differs from Y⁰ and Y^C in causing a severe veinal necrosis reaction in tobacco and milder symptoms in most potato cultivars.

PVY⁰ is the most widespread strain. The Y^N strain commonly occurs in Europe, parts of Africa, and South America and has recently been found in Canada. The Y^C strain is found in Australia, India and in some parts of Europe (8).

PVY symptoms in potato vary widely with the virus strain, potato cultivar and time of infection (8). The typical symptoms caused by the common strain, depending on the cultivar, are mottling, mosaic, yellowing of the leaflets and occasionally premature death of the plant (Fig 1). Plants may



Figure 1: Symptoms of PVY on the foliage of a potato plant.

also be stunted when infection was the result of seed transmission (8).

PVY can be transmitted by seed tubers, mechanically, core grafting, and by aphids. At least 25 species of aphids have been reported as vectors of PVY. Aphids transmit the virus in a non-persistent manner. The aphid Myzus persicae is considered one of the most efficient vectors (8).

Sigvald (40) reported that infection of older potato plants resulted in reduced tuber infection compared with similar infection of younger plants, a phenomenon he called mature-plant resistance. This study also demonstrated that late planting increased the risk of tuber infection by the virus (40).

Measures for controlling PVY in potatoes include the use of certified seed (36), chemical control of the aphids (2, 11, 35, 44), roughing (44), field inspections (44) and use of aphid-resistant potato species (10). The most efficient approach for control of PVY is preventing its arrival through a seed certification program (36).

The Wisconsin Potato Seed Certification Program, founded in 1913 (27), has been successful at controlling seed-borne diseases such as PVY (20, 38). Recently, in Wisconsin, a number of potato seed lots have been rejected due to PVY infection (German, personal communication). This is also true for seed certification agencies in other states. This

explains the importance attached to this disease by growers as indicated in the survey done by Slack (39). The reason for the increase in PVY incidence is not known; however it may be due to the popularity of a new cultivar, Russet Norkotah (German, personal communication). This cultivar is susceptible to PVY, but may only express mild symptoms (13).

Methods for diagnosis of PVY include infectivity testing on indicator plants, light microscopy, electron microscopy, comparison of physical properties, electrophoresis, and serology (8, 37). The Enzyme-Linked-ImmunoSorbent Assay (ELISA) test is the most commonly used technique in potato seed certification programs, due to its accuracy and ability to deal with large sample numbers.

POTATO EARLY DYING SYNDROME (PED). Potato Early Dying is a serious disease of potato in many regions where potatoes are grown intensively. PED is caused primarily by the fungus Verticillium dahliae (Kleb) or Verticillium albo-atrum (Reinke and Berthold) (22, 24, 32, 33). V. dahliae differs from V. albo-atrum in its ability to form microsclerotia as resting structures, while the second fungus forms melanized hyphae within infected tissues. The optimal growth temperature for V. albo-atrum is about 21°C and for V. dahliae is as high as 27°C (22, 33). In the United States, V. dahliae is more predominant in the North Central states and the Pacific

Northwest, where summer temperatures exceed 25°C. In Wisconsin, PED has been associated with V. dahliae (32).

The symptoms caused by PED are variable, and it is difficult to distinguish them from normal senescence, particularly in the early stages of the disease. In general PED is characterized by yellowing, wilting, and premature death of potato vines (6, 22, 32, 33) (Fig. 2).

Although V. dahliae is the primary pathogen of PED, other organisms have been associated with the disease. Most of the organisms that have been studied are soil-borne pathogens such as Pratylenchus penetrans, Meloidogyne hapla and Colletotricum coccodes (21, 25). Besides soil-borne organisms, other organisms such as Alternaria solani and Erwinia carotovora have been associated with PED (31, 33).

The influence and importance of those associated organisms with the primary fungus on the PED severity vary from region to region and with cultivar (33). For example, in Wisconsin, the disease is caused primarily by V. dahliae; however it has been demonstrated that the root lesion nematode Pratylenchus penetrans plays a significant role in the development of the syndrome (25, 33). Studies have shown that coinfection by the fungus and the nematode has a significant effect on the symptom expression of the disease and consequently on potato yield (25, 33).

Very few studies of the interactions between Verticillium

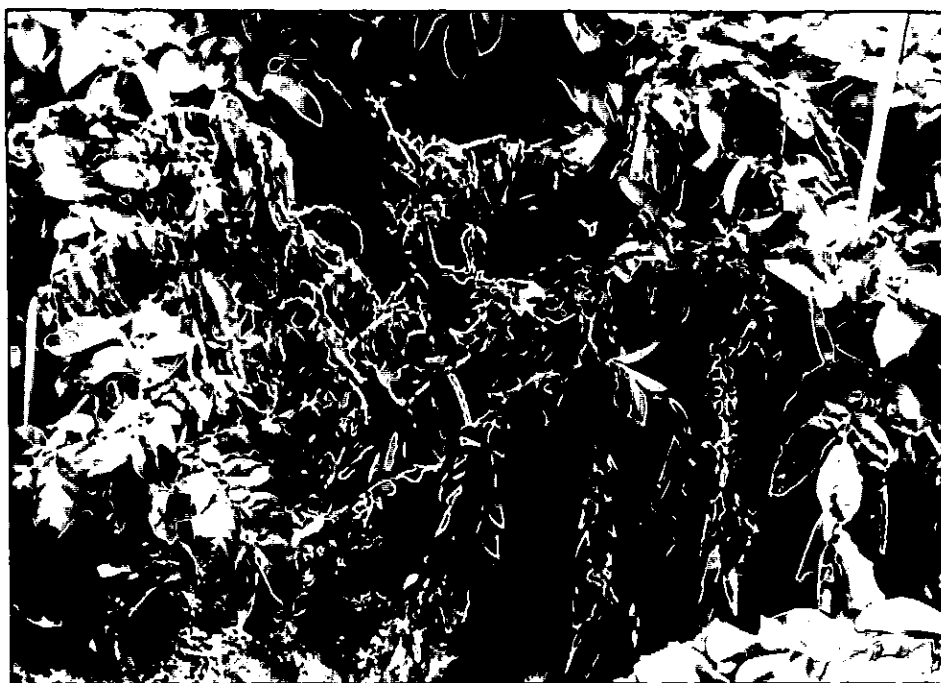


Figure 2: Symptoms of Potato Early Dying.

spp and viruses in mixed infections of plants have been reported (7, 12). The results of those studies indicated no significant interaction occurred between the combined organisms. On the other hand, interaction studies between viruses and other species of fungi have been reported in many crops. Fungus-virus interactions frequently have been found to produce either antagonistic, also called protective, or synergistic effects on the expression of one of the diseases.

Most fungus-virus interaction studies have demonstrated that the severity of a disease caused by an obligate parasite is decreased if the plant is coinfectd with a virus. For example, Wilson (43) found that bean leaves infected with Tobacco Mosaic Virus showed resistance to Uromyces phaseoli, the causal agent of bean rust. Another case of protective effect was reported by Hopen and Zeeuw (16) on cucumber. They found that plants infected by Cucumber Mosaic Virus were less susceptible to cucumber scab, caused by Cladosporium cucumerium. King et al. (19) demonstrated that the coinfection of Erysiphe polygoni with Bean Yellow Mosaic Virus on leaves of Kenland red clover also resulted in an antagonistic effect on the development of powdery mildew. Similar results were also demonstrated by Latch et al. (23) on ryegrass, in which the presence of Ryegrass Mosaic Virus suppressed the amount of crown rust, caused by Puccinia coronata. Kalra et al. (18) also found that the interaction

between Phytophthora infestans and Potato Virus X (PVX) or PVY resulted in a protective effect against the fungus. Contrary to the results that have been presented regarding coinfection of obligate fungi and viruses, Damsteegt and Ronde (5) reported recently that no evidence was found for an interaction between downy mildew and Maize Streak Virus (MSV). MSV neither increased nor decreased the susceptibility to downy mildew.

A large number of studies have demonstrated that the disease severity of those diseases caused by facultative-saprophytic fungi is usually increased if the plant is coinfecting with a virus (1, 3, 9, 17, 26, 28, 30, 34, 41). One of the examples of synergistic effect between a fungus and viruses was demonstrated by Bateman (1) on cucumber plants. Bateman found that plants infected by Cucumber Mosaic Virus (CMV) were highly susceptible to a small quantity of Rhizoctonia inoculum (1). Another study, conducted by Farley and Lockwood (9), showed that root rot caused by Aphanomyces euteiches or Fusarium solani f. pisi was more severe in Miragreen pea plants infected by Pea Mosaic Virus (PMV), Alfalfa Mosaic Virus (AMV), Bean Yellow Mosaic Virus (BYMV) or Pea Enation Mosaic Virus (PEMV). Synergism was also demonstrated by Nitzany (28) in a study on cucumber plants, in which higher mortality rates were observed when Pythium ultimum and CMV were combined. Another case, reported by

Thanassouloupoulos (41), showed that wilt symptoms on tomatoes, caused by Verticillium albo-atrum, were enhanced in the presence of Tobacco Mosaic Virus (TMV).

Given that, in Wisconsin, PED is the most common disease; the acreage of Russet Norkotah cultivar has been increasing; and PVY incidence has also increased it appears likely that both diseases may infect a single plant at the same time.

Interaction between PVY and Verticillium dahliae in the potato or any other crop has not been reported.

The present study was conducted to investigate the relationship between PVY and V. dahliae on the severity of PED and on potato yield, as described in chapter one. In addition, a complementary experiment evaluated the effect of the timing of plant infection by PVY on the infection of progeny tubers. This second experiment is described in chapter two.

REFERENCES CITED

1. Bateman, D. F. 1961. Synergism between Cucumber Mosaic Virus and Rhizoctonia in relation to Rhizoctonia damping-off of cucumber. *Phytopathology* 51:574-575 (Abstr.).
2. Bell, A. C. 1989. Use of oil and pyrethroid sprays to inhibit the spread of potato virus Y^N in the field. *Crop. Prot.* 8:37-39.
3. Beute, M. K., and Lockwood, J. L. 1968. Mechanism of increased root rot in virus infected peas. *Phytopathology* 58:1643-1651.
4. Cockerham, G. 1970. Genetical studies on resistance to potato viruses X and Y. *Heredity* 25:309-348.
5. Damsteegt, V. D., and Ronde, M. R. 1993. Interactions between Maize Streak virus and Downy mildew fungi. *Plant Dis.* 77:390-392.
6. Davis, J. R. 1981. Verticillium wilt of potato in southeastern Idaho. *Univ. Idaho Curr. Inf. Ser.* 564.
7. Davis, J. R., and Allen, T. C. 1984. Relationships of defined PVX infection levels to Verticillium wilt, yield, and quality of the Russet Burbank potato. *Am. Potato J.* 61:669-682.
8. De Bokx, J. A., and Huttinga, H. 1981. Potato virus Y. CMI/AAB Descriptions of Plant Viruses N.242. 6 pp.
9. Farley, J. D., and Lockwood, J. L. 1964. Increased susceptibility to root-rots in virus-infected peas. *Phytopathology* 54:1279-1280.
10. Gibson, R. W. 1971. Glandular hairs providing resistance to aphids in certain wild potato species. *Ann. Appl. Biol.* 68:113-119.
11. Gibson, R. W. 1986. Investigations into how cypermethrin controls the spread of Potato Virus Y by aphids. *Brighton-Crop-Prot-Conf-Pests-Dis.* 3:997-1000.
12. Goodell, J. J., Powelson, M. L., and Allen, T. C. 1982. Interrelations between Potato Virus X, Verticillium dahliae and Colletotrichum atramentarium in potato. *Phytopathology* 72:631-634.
13. Gurmen, D., Weis, G. G., et al. 1991. Commercial

- vegetable production. A3422. University of Wisconsin Extension. Madison, Wisconsin. p. 64-77.
14. Glanders, P., and Campbell, C. E. 1988. Effect of Potato Virus Y on the yield of potato cultivar Morene. Tests of Agrochemicals and Cultivars. Ann. Appl. Biol. (Supplement). 9:112.
 15. Hollings, M. 1981. Potyvirus group. CMI/AAB Descriptions of Plant Viruses N.245. 3 pp.
 16. Hopen, H. J., and Zeeuw, D. J. 1962. Reduction of susceptibility to cucumber scab by Cucumber Mosaic Virus. Plant Dis. Rep. 46:93-97.
 17. Jones, R. A. C., Fribourg, C. E., and Slack, S. A. 1981. Potato Virus and Virus-like diseases, Set No. 2. Eds O. W. Barnett and S. A. Tollin. South Carolina: Clemson University. 59pp.
 18. Kalra, A., Grover, R. K., and Rishi, N. 1989. Interactions between Phytophthora infestans and Potato Virus X and Y in Potato. J. Agric. Sci., Camb. 112:33-37.
 19. King, L. N., Hampton, R. E., and Diachun, S. 1964. Resistance to Erysiphe polygoni of red clover infected with Bean Yellow Mosaic Virus. Science 146:1054-1055.
 20. Kostichka, C. 1988. Seed potato certification history and intent. The Badger Common Tater. Nov. p. 40-41.
 21. Kotcon, J. B., Rouse, D. I., and Mitchell, J. E. 1985. Interaction of Verticillium dahliae, Collectotrichum coccodes, Rhizoctonia solani and Pratylenchus penetrans in early dying syndrome of Russet Burbank potatoes. Phytopathology 75:68-74.
 22. Krikun, J., and Orion, D. 1979. Verticillium wilt of potato: importance and control. Phytoparasitica 7:107-115.
 23. Latch, G. C., and Potter, L. R. 1977. Interaction between crown rust (Puccinia coronata) and two viruses of ryegrass. Ann. Appl. Biol. 87:139-145.
 24. Mace, M. E., Bell, A. A., and Beckman, C. H., eds. 1981. Fungal Wilt Diseases of Plants. Academic Press, New York. 640 pp.

25. MacGuidwin, A. E., and Rouse, D. I. 1990. Role of Pratylenchus penetrans in the Potato Early Dying Disease of Russet Burbank potato. *Phytopathology* 80:1077-1082.
26. Magyarosy, A. C., and Hancock, J. G. 1974. Association of virus-induced changes in rhizosphere microflora and hypocotyl exudation with protection to Fusarium stem rot. *Phytopathology* 64:994-1000.
27. Muilward, J. G. 1915. Potato seed certification in Wisconsin. Madison, Wis: Agricultural Experiment Station of the University of Wisconsin. 11 pp.
28. Nitzany, F. E. 1966. Synergism between Pythium ultimum and Cucumber Mosaic Virus. *Phytopathology* 56:1386-1389.
29. Powelson, M. L. 1985. Potato early dying disease in the Pacific Northwest caused by Erwinia carotovora and Erwinia carotovora pv. atroseptica. *Am. Potato J.* 62:173-176.
30. Pratt, R. G., Knight, W. E., and Barnett, O. W. 1981. Disease interactions of Bean Yellow Mosaic Virus and Phytophthora species in arrowleaf clover. *Phytopathology* 71:900.
31. Rahimian, M. K., and Mitchell, J. E. 1984. Relationship of Verticillium dahliae and Erwinia carotovora pv. carotovora in the early dying disease of potato. *Phytopathology* 74:327-330.
32. Rowe, R. C. 1983. Early dying - east and west. *Am. Veg. Grow.* 31:8-10.
33. Rowe, R. C., Davis, J. R., Powelson, M. L., and Rouse, D. I. 1987. Potato early dying: Causal agents and management strategies. *Plant Dis.* 71:482-489.
34. Schroth, M. N., and Teakle, S. D. 1963. Inference of virus and fungus lesions on plant exudation and chlamydospore germination of Fusarium solani f. sp. phaseoli. *Phytopathology* 53:610-612.
35. Shands, W. A. 1977. Control of aphid-borne potato virus Y in potatoes with oil emulsions. *Am. Potato J.* 54:179-187.
36. Shepard, J. F., and Claflin, L. E. 1975. Critical analyses of the principles of seed potato certification. *Annu. Rev. Phytopathology* 13:271-293.

37. Shukla, D. D., Thomas, J. E., Mckein, N. M., Tracy, S. L., and Ward, C. W. 1988. The coat protein of potyvirus. 4. Comparison of biological properties, serological relationships, and coat protein amino acid sequence of four strains of potato virus Y. Arch. Virol. 102:207-219.
38. Slack, S. A., and Darling, H. M. 1986. The seed potato program. Pages 175-184 in: With one foot in the furrow: a history of the first 75 years of the Department of Plant Pathology at the University of Wisconsin-Madison. P. H. Williams, and M. Marosy, eds. Kerdall/Kunt Pub. Co., Dubuque, Iowa.
39. Slack, S. A. 1992. A look at Potato Leafroll Virus and PVY: past, present and future. Valley Potato Grower. Dec. p. 35-39.
40. Sigvald, R. 1984. The relative efficiency of some aphid species as vectors of Potato Virus Y⁰. Potato Research 27:285-290.
41. Thanassouloupoulos, C. C. 1976. Symptom expression of tomato wilt fungi Verticillium and Fusarium as affected by the presence of Tobacco Mosaic Virus. Phytoparasitica 4:137-140.
42. Turpen, T. 1989. Molecular cloning of a Potato Virus Y genome: nucleotide sequence homology in non-coding regions of potyviruses. J. Gen. Virol. 70:1951-1960.
43. Wilson, E. M. 1958. Rust-TMV cross protection and necrotic-ring reaction in bean. Phytopathology 48:228-231.
44. Wilson, C. R., and Jones, R. A. C. 1990. Virus content of seed potato stocks produced in a unique seed potato production scheme. Ann. App. Biol. 116:103-109.

CHAPTER ONE

Interaction between Verticillium dahliae
and Potato Virus Y in potato

INTRODUCTION

Potato early dying (PED), caused primarily by Verticillium dahliae, is among the most limiting factors in potato production (11, 18, 19). The disease syndrome is made worse by interactions between V. dahliae and other pathogens of potato. The synergistic interaction between Pratylenchus penetrans, the root lesion nematode, and V. dahliae has been studied thoroughly (10, 13, 14, 20). Other pathogens reported to interact with V. dahliae include root knot nematode (12) and Erwinia carotovora (17).

Potato virus X (PVX) is the only virus that has been studied in relation to V. dahliae and PED. The results of that research showed that PVX and V. dahliae produce independent effects on symptom expression and yield when combined (4, 7). However there are several other important virus diseases of potato that may interact with the fungus. Potato Virus Y (PVY) is a potentially serious disease agent that causes mosaic symptoms and stunting of potato growth (5).

The present study was conducted to evaluate the

interactive effect of PVY and V. dahliae on the severity of PED, and potato yield and tuber quality.

MATERIALS AND METHODS

Seed source. The cultivars Russet Norkotah and Russet Burbank were used in field experiments at the Hancock Research Station in central Wisconsin, in 1991 and 1992. Russet Burbank is moderately susceptible to PVY and V. dahliae, while Russet Norkotah is highly susceptible to V. dahliae and moderately susceptible to PVY infection.

Foundation seed obtained from the UW Lelah Starks Elite Foundation Seed Potato Farm was used. This seed had been tested for PVY. No PVY infected tubers were detected in the testing. In addition to the foundation seed, virus infected seed obtained by saving tubers from mechanically inoculated plots in 1991 was used to establish specific tuber infected treatments in 1992.

Experimental design. In 1991 the experiment was designed as a Randomized Block Design (RBD) with five replicates and six treatments. Treatments were randomly assigned to plots within each block. The experiment included the following treatments: PVY foliar infection, fungus in low concentration, fungus in high concentration, a combination of PVY foliar infection with

the fungus in low concentration, a combination of PVY foliar infection with the fungus in high concentration, and control. The experimental area was divided into an even number of blocks with a none potato border around each block. Each cultivar was assigned randomly to each pair of blocks. In 1992, some alterations were made due to space limitations and to the introduction of three additional treatments involving PVY infected tubers. In this year the experiment was conducted as a completely randomized design, with five replicates of each of nine treatments. The three extra treatments included in 1992 were the following: PVY infected tubers, a combination of PVY infected tubers with the fungus in low concentration, and a combination of PVY infected tubers with the fungus in high concentration.

Plot Establishment. Prior to planting, the soil was fumigated with metam-sodium and fertilized with 0-0-50 NPK. Tubers were cut and planted mechanically, with the exception of PVY infected tubers. PVY infected tubers used in 1992 were cut and planted by hand to avoid excessive contamination of the mechanical planter. The fields were planted on May 1 and May 3 in 1991 and 1992, respectively. Each experimental unit consisted of four rows. Data were collected from the center two rows only. The distance between rows was 91 cm and the seed pieces were planted 30 cm apart in the row. The field

plots were irrigated with a center pivot system. The cultural practices used in all plots are shown in Table 1.

PVY Inoculum and PVY Field Inoculation. PVY⁰ was maintained on potato plants in the greenhouse. Detached leaves from a single infected plant were ground with phosphate buffer solution (PBS) pH=7.4, in a proportion of 1:10 of plant tissue weight: buffer volume. The extract was filtered with cheese cloth and the resulting solution was gently rubbed over all the foliage of the propagative plants (Gold Rush potato cultivar) when they were three weeks old. About 35 days after inoculation, the infected propagative plants were used as the source of PVY inoculum for the field experiments.

Prior to inoculation, the foliage of the plants was dusted with carborundum. Four complete leaves on each plant were randomly selected and inoculated using a paint brush. The inoculum was chilled over ice in a cooler until it was applied to the leaves.

Several tests were performed to reconfirm the identity of the virus. The virus was first propagated in Nicotiana tabacum c.v. Xanthi and then purified according to the modified procedure described originally by Yang, Reddick and Slack (24). The purified preparation was scanned in the spectrophotometer to determine virus concentration and was also analyzed by electron microscopy, infectivity test

Table 1: Main Cultural practices applied in 1991 and 1992

Type and time of practice	Implement used or product applied	
	1991	1992
<u>Tillage</u>		
preplant		
post emergence	disk + plow hilling	disk + plow hilling
<u>Fertilization</u> ⁽¹⁾		
before planting	0-0-50: 444 Kg/ha	0-0-50: 222 Kg/ha
at planting (May 1st)	5-10-30: 721 Kg/ha	5-10-30: 666 Kg/ha
mid-May	33-0-0: 333 Kg/ha	34-0-0: 222 Kg/ha
mid-june	33-0-0: 333 Kg/ha	34-0-0: 433 Kg/ha
<u>Herbicide</u>		
post emergence	Lorox	Lorox
mid September ⁽²⁾	Diquat & oil	Diquat
<u>Insecticide</u>		
early June	Pydrin	Furadan
mid June	-	Thiodan
early July	Pydrin	Dimethoate
late July	Thiodan	Asana
<u>Fungicide</u>		
June-September ⁽³⁾	Bravo	
July ⁽⁴⁾		Bravo
		Dithane M-45
August-September ⁽⁵⁾		Maneb & Zinc
		Bravo

(1) The type of fertilizer indicates the equivalent N-P-K.

(2) Vein killing.

(3) Applied weekly from mid June till early September.

(4) In July, Bravo applied only the first week.

(5) Maneb & Zinc applied only the first week of August.

(Appendix III) and double antibody sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA). Negative staining electron microscopy was used to confirm the morphology of the virus particle (Appendix III). The virus infectiveness was evaluated based on the number of chlorotic local lesions produced on the indicator plant, Chenopodium amaranticolor. The indicator plant was mechanically inoculated with the purified preparation using a series of dilutions. The number of lesions produced was then recorded (Appendix III). A series of dilutions of the purified virus were also analyzed by DAS-ELISA. The reagents for the assay were provided in kits by Agdia Inc. (Elkhart, Indiana) and the assay was performed according to the manufacture's instructions. Absorbance (A_{409}) values were measured in a Bio-tek multiscan photometer. Samples from known infected and healthy potato plants were included on each plate. The criteria established for negative-positive threshold was based on the mean value of the negative control and its standard deviation. A positive result was one with an optical density value equal to or higher than 0.13, obtained from the sum of the mean of the negative control plus four times its standard deviation (23).

The inoculum of PVY used in the field was also quantified using the DAS-ELISA and infectivity test on C. amaranticolor.

Vd Inoculum and Soil Infestation. In 1991, microsclerotia

were produced on 10% PDA. A suspension of the microsclerotia was prepared by mixing the contents of the plates with water in a waring blender. The inoculum concentration was adjusted to 4×10^4 propagules per milliliter.

The suspension was poured over each pre-selected plot by hand. Each plot received 500 ml or 100 ml of suspension, which were designated as high and low concentrations of inoculum, respectively.

In 1992, inoculum of V. dahliae was produced by growing the fungus on moistened, sterile rye seed, in mason jars at room temperature in the dark. After 4-6 weeks of incubation the rye seeds were air dried and ground in a Wiley mill.

The inoculum of the fungus contained in bags was mixed with an approximately equal volume of soil and subsequently applied by hand in the two middle rows of the selected plots. In each plot 250 g and 1,000 g were applied as low and high concentrations, respectively. Then, the inoculum was incorporated to a depth of 15 cm with a rototiller.

Soil Assay. Soil samples were taken from each of the individual plots and assayed for V. dahliae using the soil-dilution plating technique described by Nicot and Rouse (16). This technique uses 10-g subsamples of air-dried soil. The subsamples were mixed with 100 ml water in 250-ml Erlenmeyer flasks. One ml aliquots of the soil suspension were taken

from the flasks and plated on a selective medium for V. dahliae. Plates were scored after two to three weeks of incubation by counting the number of colonies that formed typical microsclerotia (Fig. 3). The estimate of soil inoculum in a sample was expressed as an average number of colony-forming units (cfu) of V. dahliae per gram of dry soil.

Plant Assay For Vd. The vascular colonization of potato plants by the fungus was quantified twice during the season for Russet Norkotah and three times for Russet Burbank. Three main stems were randomly selected from the sampling area in each plot. Four centimeter lengths of stem were cut from near the base of the plant. This stem segment was surface sterilized for 30 seconds in 10% clorox. One end of the stem segment was then cut to provide a clean surface. The segments were wrapped in parafilm and placed in a plant press. The sap was extracted from the end of the stem segment. A pipet was used to collect 0.1ml of the expressed sap which was then plated on a selective medium for V. dahliae. After two to three weeks of incubation, the number of colony forming units per plate were counted. The readings were transformed to colony forming units per milliliter of sap (cfu/ml of sap).

Plant Assay for PVY on the Foliage. The virus content on the foliage over the growing season was determined by DAS-ELISA

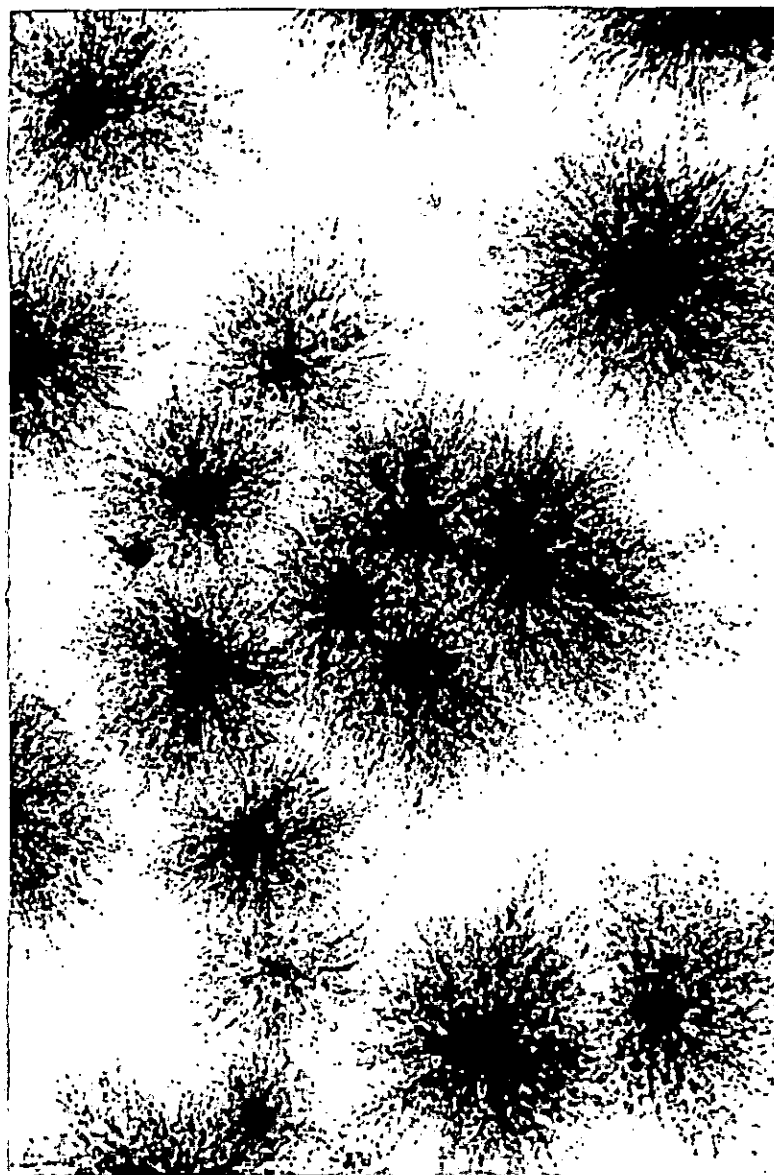


Figure 3: Microsclerotia colonies of V. dahliae.

following the procedure provided by AGDIA Inc. Samples of the foliage were collected twice during the season, 45 and 75 days after planting (dap). An extra sampling was done about 110 dap only for Russet Burbank. In each plot three stems were randomly selected and bulked in the same bag. Then, twenty leaflets from each stem were selected randomly and grouped into a single pile. Leaflet discs were cut from the pile with a sterile cork borer #9. After each cut the cork borer was sterilized in a solution of 1% of Sodium Dodecyl Sulphate-Alconox. Then, the sap from the leaflet discs was extracted in a mechanical tissue grinder. Two drops of the extracted sap were collected into a microcentrifuge tube containing 1 ml of phosphate buffer solution, corresponding to a ratio of about 1:10 of volume of sap and volume of extract buffer. A micropipet was used to collect 100 μ l of the sample which was analyzed by DAS-ELISA.

Plant Assay for PVY on the Tubers. Ten tubers were randomly selected from each plot and assayed individually for PVY. This assay was done on the sprouts. Tubers were treated with Rindite to produce earlier and larger sprouts. Rindite is an organic solution containing ethylene chlorohydrin, dichloroethane and carbon tetrachloride in a proportion of 7:3:1, respectively. Four to five sprout tip pieces, approximately 0.05 grams of tissue, were cut from each tuber

with a sterile scalpel. The sprout pieces were then placed immediately in a microcentrifuge tube containing 0.5 milliliters of enriched sample extract buffer solution (Phosphate buffer solution containing Tween 20, egg albumin, polyvinyl pyrrolidone and sodium sulfite), equivalent to a dilution ratio of about 1:10 of tissue weight and extract buffer volume. Then the tissue was ground with a pellet pestle. A micropipet was used to collect 100 microliters of the sample which was then analyzed by DAS-ELISA.

Sequential rating of disease severity. During the growing season disease development was monitored by visually scoring disease symptoms. PVY symptoms were evaluated on three dates in 1991 based on a two level scale of 0 = no symptoms and 1 = PVY symptoms (mosaic, yellowing, mottling or necrosis on the foliage). In 1992, PVY symptoms were assessed on ten dates using a scale of 0 = no symptoms, 1 = < 25% of the sampling area show PVY symptoms, 2 = 25-50%, 3 = > 50% and 4 = dead plants. Symptoms of PED were assessed on three and ten dates in 1991 and 1992, respectively, using a scale of 0 = no symptoms, 1 = < 25% of the sampling area showing yellowing, wilting or necrosis, 2 = 25-50%, 3 = > 50% but none of the plants are dead, 4 = dead plants. Disease scoring started when the first evidence of PVY and PED symptoms appeared in the field. The assessment was based on the whole sampling

area, not on an individual plant.

Canopy development. Light intercepted by the canopy was measured with a pyranometer (LICOR, Lincoln, Nebraska). Data were collected by taking three replicate readings within each plot above and below the canopy.

In 1992, leaf area was measured on the leaves of stems collected for sap squeezing. Detached leaflets were measured with an electronic leaf area meter (LICOR, Lincoln, Nebraska). This device uses a method of rectangular approximation with a resolution of 1 mm² (9).

Potato yield. Potato tubers were harvested and graded mechanically into three categories: A, B and Cull. The A's consisted of tubers with a diameter greater than 4 ounces. Tubers less than 4 ounces were B's. Tubers that were damaged were culled. Damage signs included rots, scab, mechanical damage and malformations. Specific gravity was measured using a potato hydrometer.

Statistical analysis. The results were analyzed by using the general Linear Models procedure (PROC GLM) of the Statistical Analysis System (SAS) (21). The standard errors and the Fisher's least significant difference (LSD) for multiple comparisons were calculated based on the procedure described

by Milliken and Johnson (15). The summaries are given in table or graph form. All graphics were plotted by the 'Cricket Graph' program.

RESULTS

Effect of PVY alone on viral symptom expression. In 1991, mosaic symptoms appeared on the leaflets about twenty days after foliar inoculation (50 dap). As the season progressed symptoms became more severe, although data were not collected to quantitate severity through time. In 1992, very mild mosaic symptoms were first observed 10 and 40 days after inoculation (40 and 70 dap) on Russet Burbank and Russet Norkotah, respectively (Fig. 4). Disease severity did not appear to increase after initial symptoms were observed. Less than 25% of the plants showed viral symptoms, although all plants within the sampling area had been inoculated.

Viral symptoms, including mottling and crinkling of the foliage, were observed in treatments with virus infected tubers about 20 dap, corresponding to 50% of plants emerged (Fig. 4). In those treatments virus symptoms became progressively more severe, showing severe mosaic over the next 6 and 7 weeks for Russet Norkotah and Russet Burbank, respectively. Symptom severity then leveled off or declined. Plants infected by PVY through tubers were stunted.

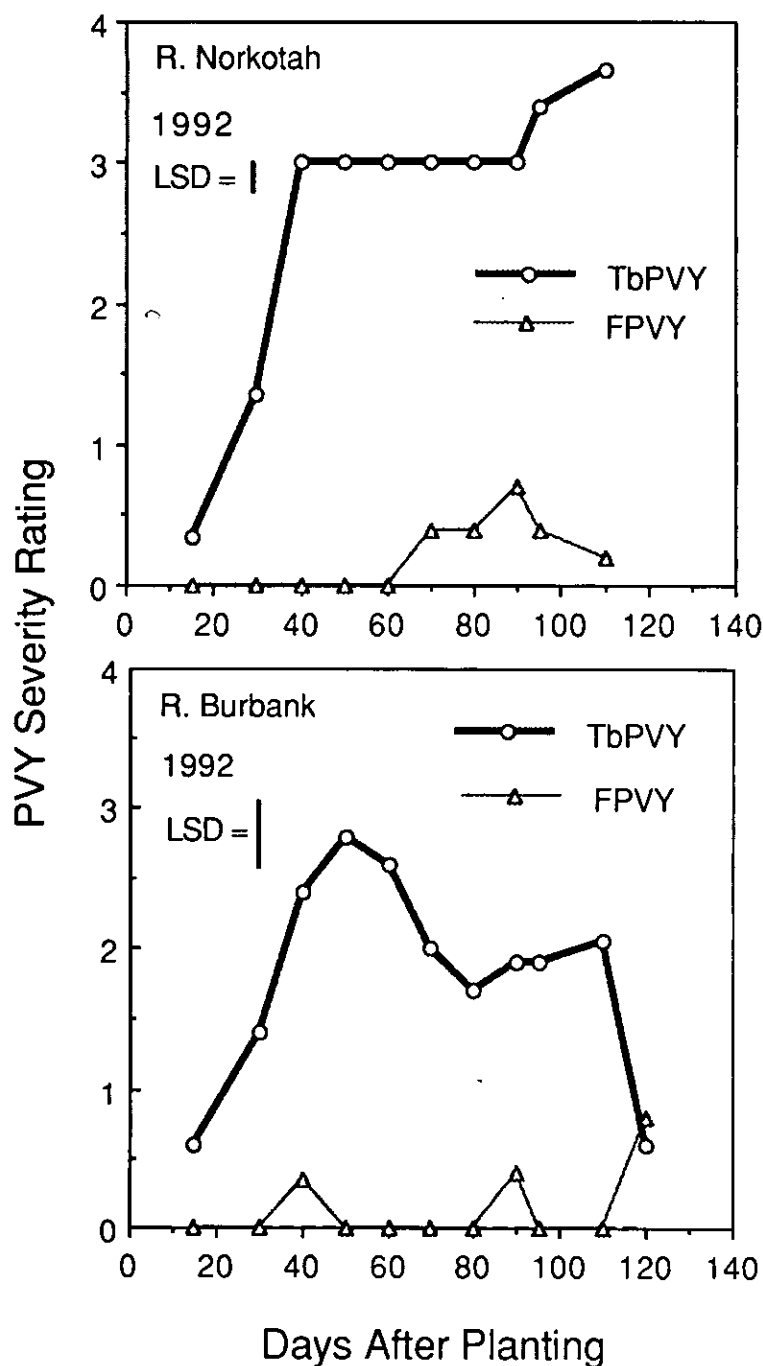


Figure 4: PVY disease severity rating (0-4 scale) on the foliage of Russet Norkotah and Russet Burbank potato plants infected by PVY alone. The treatments are: FPVY = PVY foliar infection and TbPVY = PVY tuber infection).

Effect of *Verticillium dahliae* alone on PED severity. In 1991, mild symptoms of early dying, consisting of yellowing of lower leaves, were observed about 45 dap in treatments with V. dahliae in low concentration in Russet Norkotah and high concentration in Russet Burbank. Disease severity remained generally very low through the season, with a disease rating average of 0.5. The low disease intensity observed in this year was consistent with the results of the soil assay (Appendix II). The fungus was not recovered in treatments with the fungus at high and low concentrations in Russet Norkotah and Russet Burbank, respectively. In those treatments where the fungus was recovered, it was an average of 8 cfu/g of soil.

In 1992, in both cultivars, PED symptoms appeared 60 days after planting in treatments with the fungus alone. No significant difference was observed regarding PED symptom expression, when the two concentrations of the fungus were compared (Fig. 5). The results of the soil assay were highly variable. The results on Russet Norkotah did not show a significant difference between the two levels of the fungus. The average number of propagules recovered from soil was 13 and 17 on low and high concentrations, respectively. However, in Russet Burbank the average number of propagules recovered from the soil was significantly different between the two levels of the fungus. The average number of cfu/g of soil was

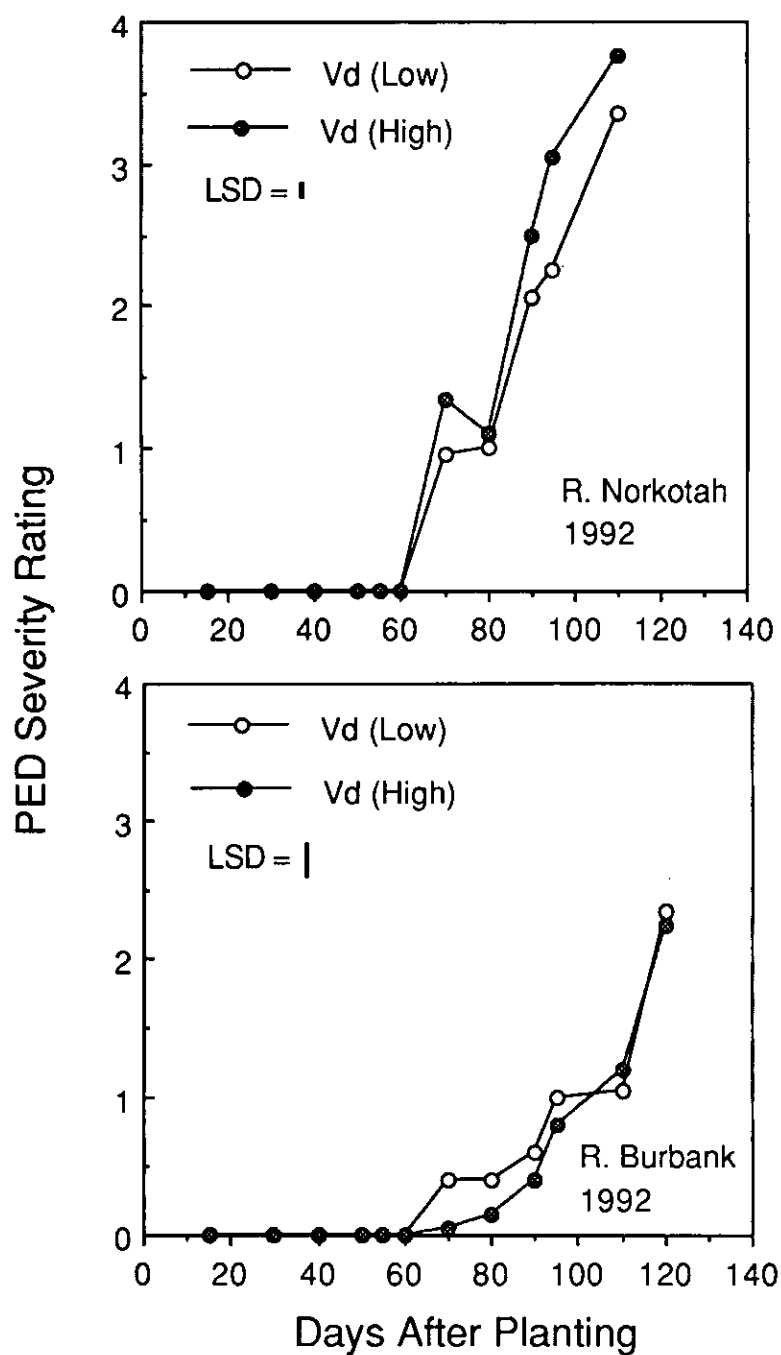


Figure 5: PED disease severity rating (0-4 scale) on Russet Norkotah and Russet Burbank potato plants infected by *Verticillium dahliae* alone. The treatments are: Vd(Low) = soil infested with fungus in low concentration and Vd(High) = soil infested with fungus in high concentration).

22 and 35 for low and high concentration, respectively.

Effect of *Verticillium dahliae* on PED severity in the presence of PVY. In both years there was no effect of the foliar infection by PVY on the severity of PED.

The effect of tuber infection was highly significant on the development of PED, in both cultivars. Plants coinfectd with PVY and *V. dahliae* started to show PED symptoms about ten days earlier in the season compared to the treatments that had only the fungus (Fig. 6). The first symptoms observed in most of the plants infected with both pathogens were yellowing, chlorosis and/or crinkle of the leaflets. About 35 days after planting, the most predominant symptom was mosaic that prevailed until the plants started to wilt. The presence of PVY accelerated the expression of PED and increased its severity (Fig. 6). Also, it was found that when PVY was present there was a significant difference in the severity of PED between the two levels of the fungus. The higher the inoculum density the higher the severity of PED in the presence of the virus (Fig. 6).

Effect of the virus on vascular colonization by the fungus.

Vascular colonization by the fungus, as measured by sap plating, was significantly increased in the presence of the virus. In Russet Norkotah this effect was observed about 45

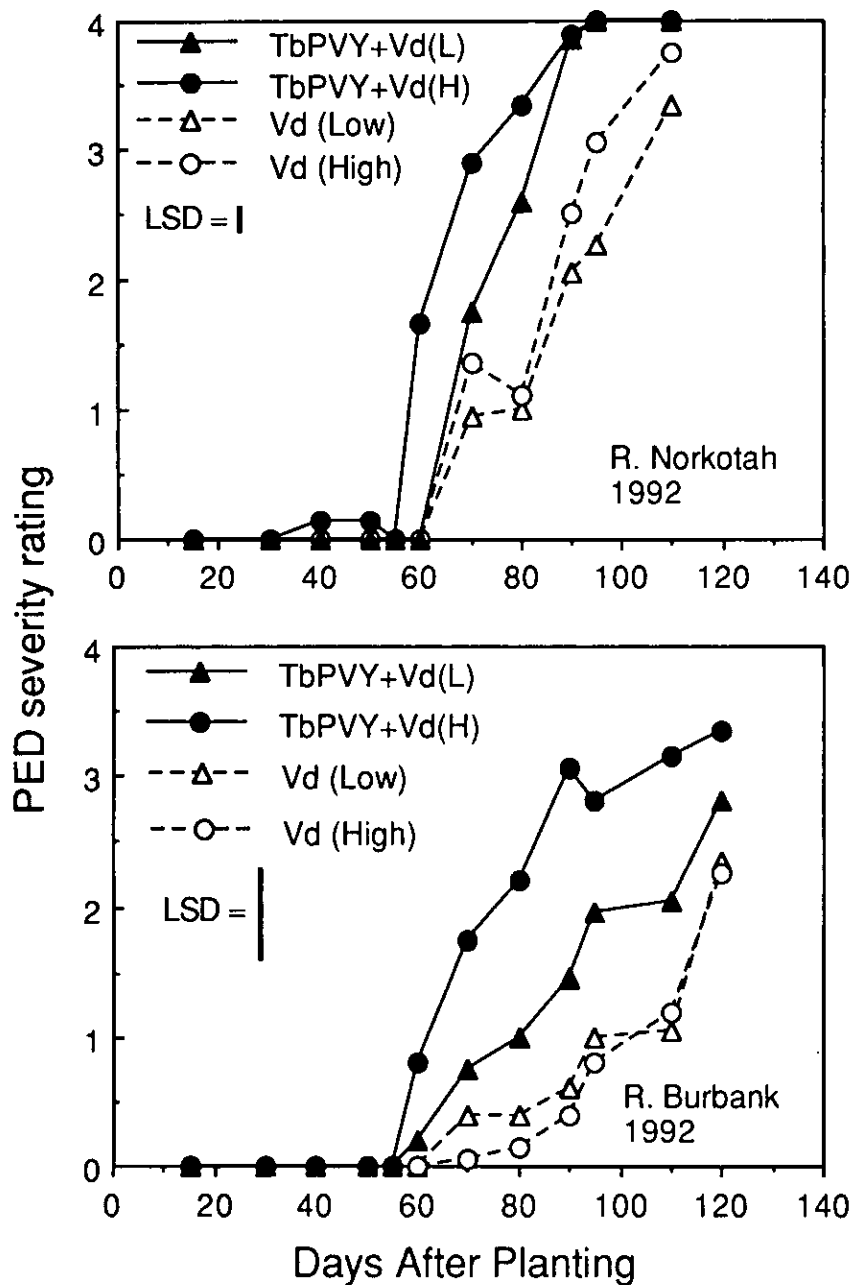


Figure 6: Effect of *Verticillium dahliae* on the severity of PED in the presence of PVY for Russet Norkotah and Russet Burbank potato plants. The treatments are: Vd(Low) = soil infested with fungus in low concentration, Vd(High) = soil infested with fungus in high concentration, TbPVY+Vd(L) = combination of infected tubers with fungus in low concentration, and TbPVY+Vd(H) = combination of infected tubers with fungus in high concentration.

dap and only if the fungus was in high concentration. In Russet Burbank both levels of the fungus showed a significant effect of the virus on the colonization by the fungus 70 days after planting (Table 2).

Effect of the fungus on the virus content in the plant. The results from the DAS-ELISA test on the foliage indicated that the presence of the fungus might affect the virus content. It was found that in the presence of the fungus the optical density values at 490 nm were lower, but the difference was not statistically significant (Table 3).

PVY infection on progeny tubers. The ELISA test on the sprouts of the tubers produced in 1991 indicated that the foliar infection resulted in about 60% of progeny tubers infected with PVY on Russet Norkotah and Russet Burbank cultivars. In 1992 the foliar infection resulted in a low percentage of progeny tubers infected with PVY, however the tuber infection gave about 80 and 40% of progeny tubers infected with PVY in Russet Norkotah and Russet Burbank (Fig. 7). Coinfection demonstrated that the presence of the fungus slightly decreased the proportion of progeny tubers infected with PVY (see Appendix II).

Canopy development. Readings from the light bar showed that the amount of light intercepted by the plant was affected by

Table 2: Effect of PVY tuber infection on vascular colonization by Verticillium dahliae for Russet Norkotah and Russet Burbank.

Cultivar	Inoculum	Average C.F.U./ml sap	
		(Days After Planting)	
		45	75
R. Norkotah	Vd(Low)	601	2422 (ba) ^x
	TbPVY+Vd(L)	1	1812 (ba)
	Vd(High)	47	3578 (a)
	TbPVY+Vd(H)	389	3051 (a)
	TbPVY	0	0 (c)
R. Burbank	Vd(Low)	27	205 (bc)
	TbPVY+Vd(L)	55	2005 (ba)
	Vd(High)	275	1452 (bc)
	TbPVY+Vd(H)	8	3492 (a)
	TbPVY	0	0 (c)

^x Numbers with the same letter for each cultivar (75 dap) are not significantly different at $\alpha=0.05$.

Table 3: Effect of the fungus on the virus content the foliage of Russet Norkotah and Russet Burbank.

<u>Cultivar</u>	<u>Inoculum</u>	<u>OD (490 nm)</u>	
		<u>(Days After Planting)</u>	
		<u>45</u>	<u>75</u>
R. Norkotah	Vd(Low)	0.036 (c) ^x	0.000 (c)
	TbPVY+Vd(Low)	1.267 (b)	1.781 (a)
	Vd(High)	0.000 (c)	0.000 (c)
	TbPVY+Vd(H)	1.495 (ba)	1.418 (b)
	TbPVY	1.938 (a)	1.6648 (ab)
R. Burbank	Vd(Low)	0.000 (b)	0.000 (c)
	TbPVY+Vd(L)	0.197 (ba)	0.904 (ab)
	Vd(High)	0.020 (b)	0.000 (c)
	TbPVY+Vd(H)	0.321 (ba)	0.586 (b)
	TbPVY	0.355 (a)	1.000 (a)

^x Numbers with same letter for each cultivar at 45 and 75 dap are not significantly different $\alpha=0.05$.

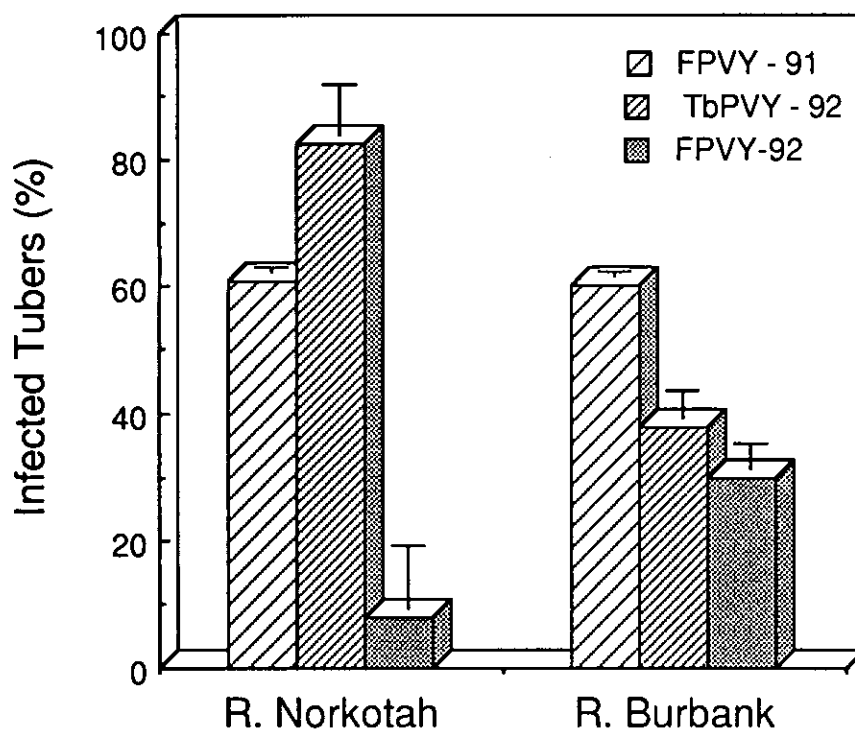


Figure 7: Percentage of progeny tubers infected by PVY, harvested from plants mechanically inoculated in 1991 (FPVY-91) and in 1992 (FPVY-92) or grown from infected tubers (TbPVY-92) for Russet Norkotah and Russet Burbank potato plants.

the presence of one or both pathogens. The light intercepted by the plant decreased progressively over time. The biggest effect of the treatments on light intercepted by the plant was observed in those treatments with a combination of PVY (tuber infection) and V. dahliae in high concentration (Fig. 8).

In 1992, the virus or the fungus or the combination of both caused a reduction in the amount of functional leaf surface (Table 4). Also, it was observed that the effect of the combination of both pathogens in the reduction of the green leaf area was higher than the effect of the virus alone (Table 4).

Effect of PVY alone on the total potato yield. The data from both years showed a significant decrease in potato yield caused by PVY alone (Fig. 9). In 1991 the yield loss caused by PVY foliar infection was about 30% and 20% in Russet Norkotah and Russet Burbank, respectively. In 1992 there was no effect of foliar infection on potato yield (Fig. 9).

The yield reduction caused by PVY infected tubers was about 50% in both cultivars (Fig. 9).

Effect of the fungus alone on total potato yield. In 1991, the fungus did not have a measurable effect on potato yield. In 1992, although two levels of Vd (low and high) were applied, there was not a significant difference between them

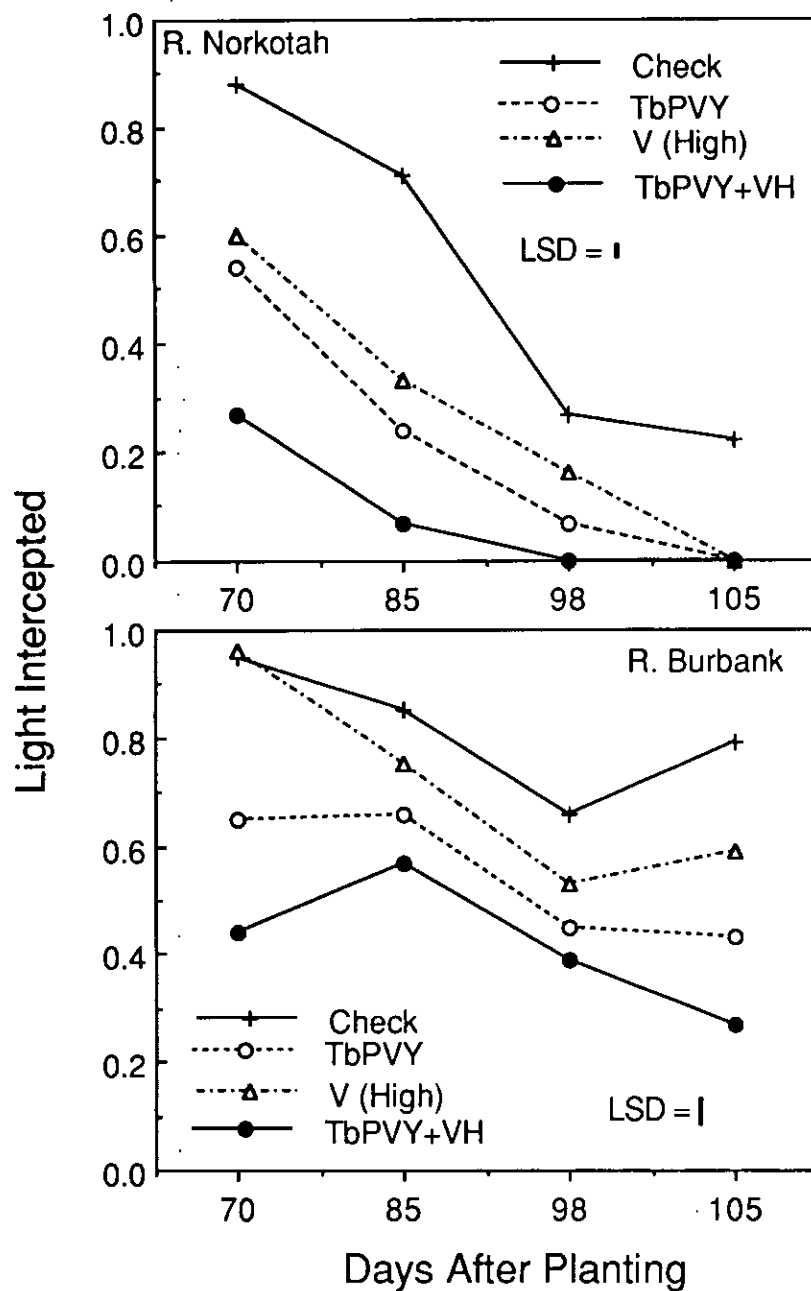


Figure 8: Proportion of light intercepted by potato plants grown under different conditions: uninfected soil and virus-free (check), or infected tubers (TbPVY), or soil infested with fungus in high concentration (V High), or a combination of both pathogens (TbPVY+VH).

Table 4: Percentage reduction of the green leaf area by the presence of PVY and Verticillium dahliae on Russet Norkotah and Russet Burbank potato plants.

<u>Cultivar</u>	<u>Inoculum</u>	<u>Green area reduction(%)</u> <u>(Days After Planting)</u>	
		<u>45</u>	<u>75</u>
Norkotah	Vd(Low)	0	45
	Vd(High)	14	43
	TbPVY	45	74
	TbPVY+VL	54	75
	TbPVY+VH	44	87
R. Burbank	Vd(Low)	0	0
	Vd(High)	0	8
	TbPVY	42	57
	TbPVY+VL	60	34
	TbPVY+VH	65	85

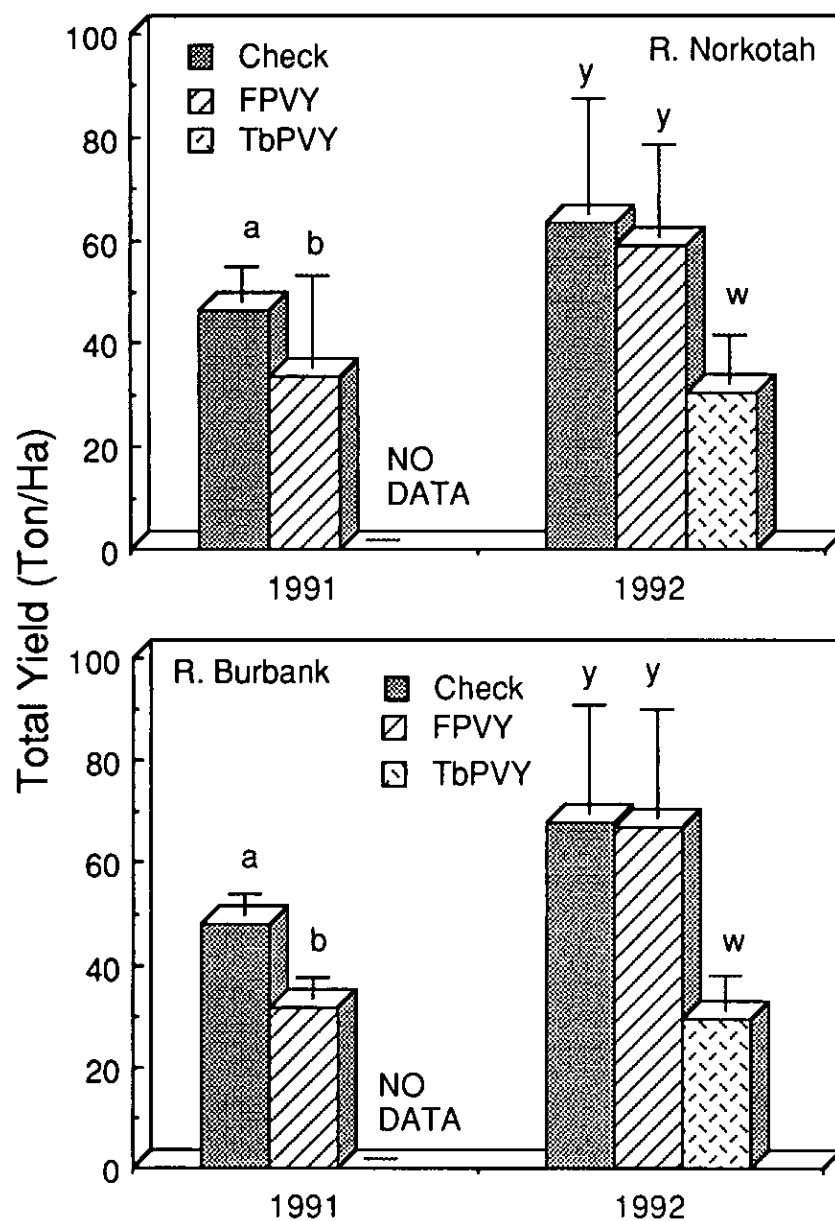


Figure 9: Effect of PVY alone on total potato yield (Ton/ha) for Russet Norkotah and Russet Burbank. The treatments are: FPVY = PVY foliar infection and TbPVY = PVY infected tubers. Symbols a,b refers to 1991 and y,w to 1992. Means with same symbol are not significant different at $\alpha = 0.05$.

at a significance level of 5%. The yield reduction due to the fungus alone was 30% and 20% on the Russet Norkotah and Russet Burbank cultivars, respectively (Fig. 10).

Effect of Vd and PVY on total potato yield. Data from 1992 indicated that the magnitude of yield loss caused by PVY tuber infection in the presence of the fungus was cultivar dependent. The combination of both pathogens reduced total yield by as much as 70% for Russet Norkotah. The observed interactive effect was additive, for both levels of the fungus. For Russet Burbank an additive effect on potato yield only occurred when the fungus was in high concentration (Fig. 11).

Effect of PVY and Vd on potato yield quality. As with total yield, the quality of potato tubers was affected in the presence of PVY alone, V. dahliae alone or both pathogens combined. Each of the pathogen treatments reduced the yield of A's tubers. However, the combination of both pathogens showed an additive effect for both levels of the fungus in Russet Norkotah and with the fungus in high concentration on Russet Burbank (Fig. 12).

Specific gravity. The specific gravity was only affected by the presence of the fungus. For Russet Norkotah, the effect

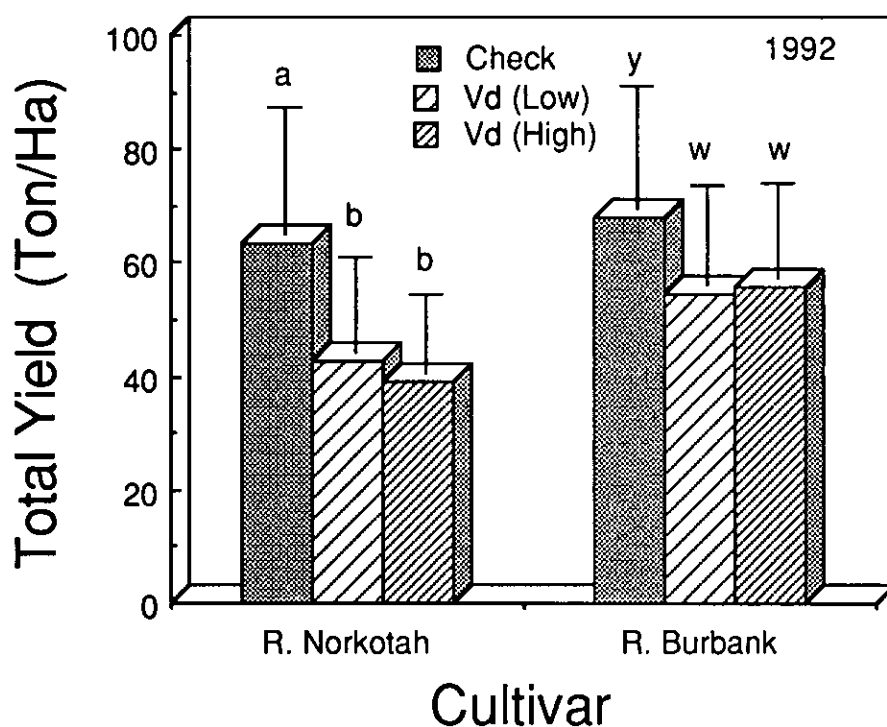


Figure 10: Effect of the fungus alone on total potato yield (Ton/ha) for Russet Norkotah and Russet Burbank cultivars. The treatments are: Check, Vd(Low) = soil infested with fungus in low concentration, Vd(High) = soil infested with fungus in high concentration. Symbols a,b refers to Russet Norkotah and y,w to Russet Burbank. Means with same letter are not significantly different at $\alpha = 0.05$.

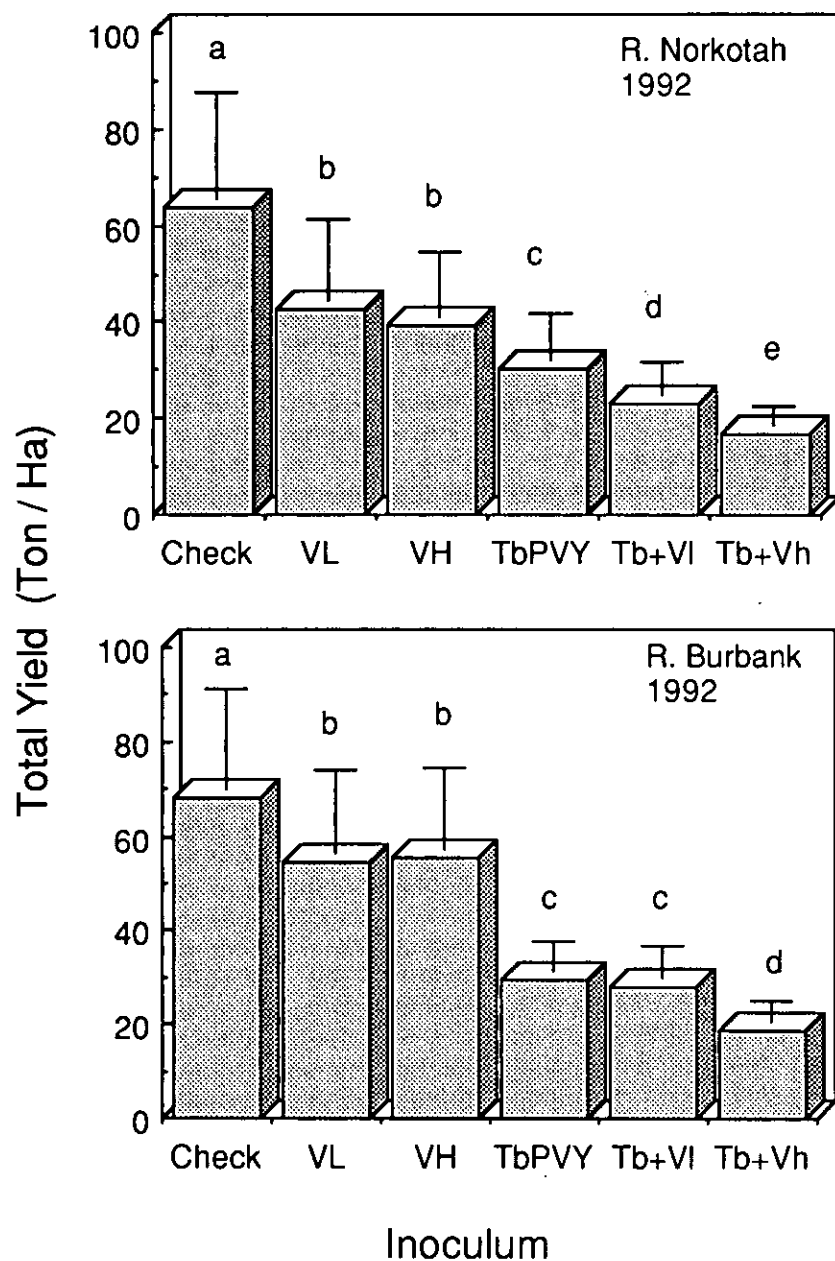


Figure 11: Effect of combination of *Verticillium dahliae* and PVY on total potato yield (Ton/ha) on Russet Norkotah and Russet Burbank. The treatments are: Check, VL = soil infested with fungus in low concentration, VH = soil infested with fungus in high concentration, TbPVY = PVY infected tubers, Tb+VL and Tb+Vh = combination of both pathogens. Means with same letter are not significantly different at $\alpha = 0.05$.

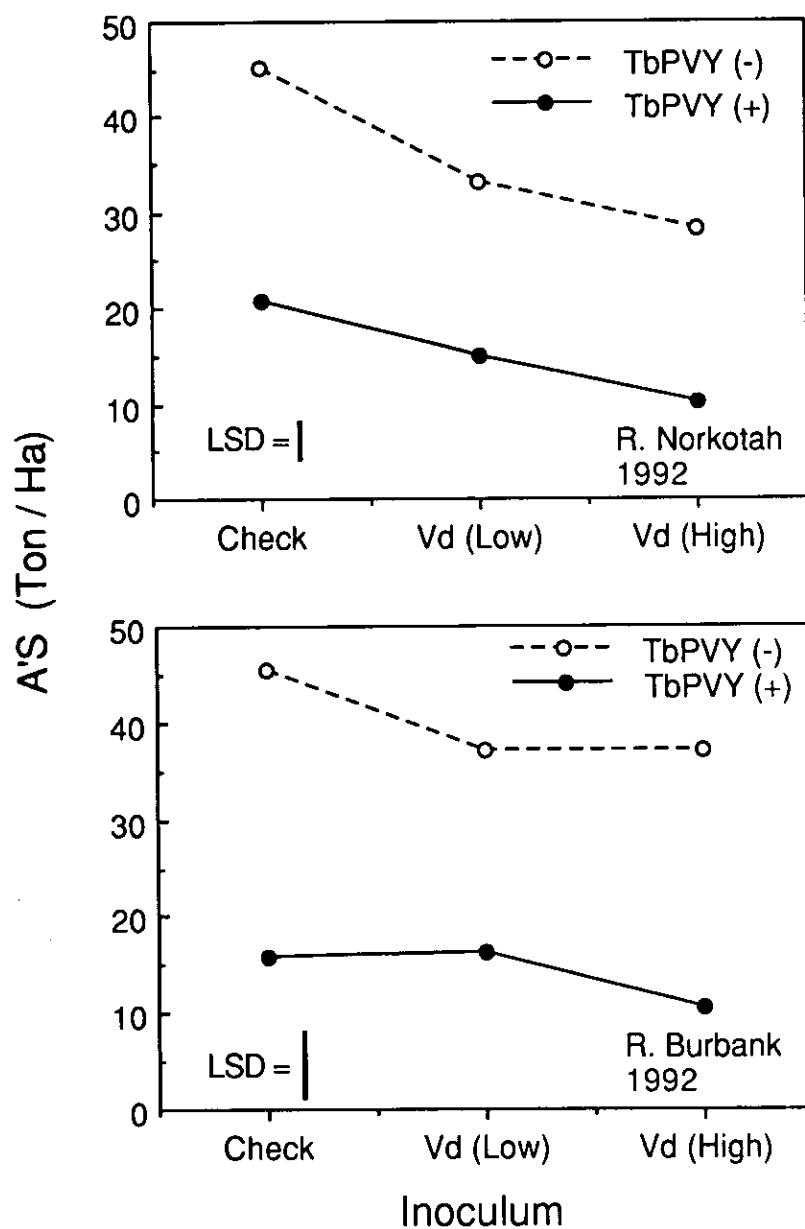


Figure 12: Effect of PVY and *Verticillium dahliae* on yield of 'A' category tubers (Ton/ha) for Russet Norkotah and Russet Burbank cultivars. The treatments are: Check, Vd(Low) = soil infested with fungus in low concentration, Vd(High) = soil infested with fungus in high concentration, TbPVY(-) = free-PVY tubers, and TbPVY(+) = PVY infected tubers.

was statistically significant at $\alpha = 0.05$, in both levels of the fungus, and for Russet Burbank only the high concentration of the fungus showed significance (Table 5). The coinfection by both pathogens did not show a significant effect on the specific gravity (Table 5).

DISCUSSION

This present study has demonstrated the importance of PVY on the development of PED in Russet Norkotah and Russet Burbank cultivars. The results showed that viral symptoms induced by PVY⁰ also vary with the time of infection. Symptoms produced by foliar infection were characterized by yellowing, mottling and moderate mosaic symptoms, whereas, tuber infection induced stunting, severe mosaic symptoms, and premature dying. Similar results regarding PVY symptoms have been reported previously (5, 8).

The reduced PVY disease severity observed in 1992 in plants mechanically inoculated was not related to the quality of the inoculum. This assumption is based on the results of the ELISA and infectivity tests of the virus inoculum. The $OD_{490\text{ nm}}$ average of the virus inoculum was 1.534. C. amaranticolor inoculated with the virus inoculum showed chlorotic lesions 6 days after inoculation (Appendix III). The results obtained in 1992 regarding foliar infection

Table 5: Effect of PVY and V. dahliae on specific gravity.

Treatment	<u>Cultivar</u>	
	R. Norkotah	R. Burbank
Check	1.0634 (b) ^x	1.0796 (y) ^x
Vd(Low)	1.0700 (a)	1.0792 (y)
Vd(High)	1.0658 (ba)	1.0726 (w)
TbPVY	1.0620 (b)	1.0748 (wy)
TbPVY+Vd(L)	1.0615 (b)	1.0738 (wy)
TbPVY+Vd(H)	1.0610 (b)	1.0714 (w)

The treatments are: Check, Vd(Low) = soil infested with fungus in low concentration, Vd(High) = soil infested with fungus in high concentration, TbPVY = PVY infected tubers, TbPVY+Vd(L) and TbPVY+Vd(H) = combination of both pathogens.

(x) Means with same letter are not significantly different at $\alpha = 0.05$.

suggest that environmental conditions, in particular low temperature, may be affected virus multiplication and subsequently the expression of viral symptoms. De Bokx and Piron (6) reported that symptom expression by PVY may be affected by many factors, including temperature. They found that potato plants infected with PVY (O) showed symptoms two weeks after inoculation when grown at high (22-26° C), but not at low temperature (10,14,18° C). Close (2) reported that the optimum temperature for PVY multiplication is approximately 28° C. In 1992, the growing season was mostly cool and frost occurred about four weeks after virus inoculation. Thus, virus multiplication may have been inhibited due to low temperature. Alternatively, inoculated leaves may have been killed by frost before the virus was translocated to other parts of the plant. This possibility is reenforced by the observation that frost damage was accentuated in those treatments with PVY.

In 1991, the lack of effect of the fungus on PED symptom expression may be attributed to the low inoculum density of V. dahliae. Among many factors, those results might be associated with the type and amount of inoculum applied in that year. The effect of V. dahliae on PED observed in 1992 suggests that above the threshold level, and when the fungus is acting alone, a higher concentration of the fungus does not affect disease severity.

PVY infected tubers increased the severity of PED for both levels of the fungus. The relationship was synergistic in both cultivars. When PVY was present, the two levels of the fungus showed a significant difference in symptom expression of PED. Beute and Lockwood (1) found that virus infected plants increased exudation of many kinds of compounds, including nutrients utilizable by fungi. Possible mechanisms of the increased susceptibility of plants infected with a virus to diseases caused by soil-borne fungi may include acceleration of spore germination and alteration of the host metabolism (3).

The results of the sap plating, added to the fact that PED symptoms occurred earlier in the season in treatments with combined infection, strongly suggest that one possible mechanism of increased severity of PED might be an acceleration of microsclerotial germination (3). This observation requires further investigation.

ELISA testing for PVY on the progeny tubers showed that plants grown from PVY infected seed (tuber infection) had a higher proportion of infected tubers compared to foliar inoculated plants (Foliar Infection). Sigvald (22) found that the later infection takes place the less virus will reach the tubers. This experiment did not include infection by PVY in later stages of plant development. However, the data on tuber infection and foliar infection at 30 days after planting are

in accord with that of Sigvald (22).

In general, the results demonstrated trends toward increased yield loss due to the presence of either pathogen. The time of PVY infection as well as cultivar had a significant effect on potato yield. Our findings are in accord with those reported by Bokx (5). On the other hand, our results suggest that the increase in fungus concentration, above the threshold level, does not affect the yield response to V. dahliae alone. The effect of the combined infection by PVY and V. dahliae on the yield of Russet Norkotah and Russet Burbank resulted in about 70% yield loss. In Russet Norkotah the effect was additive on both levels of the fungus and in Russet Burbank it was observed only at the high concentration. This increase in yield loss, when both pathogens are combined, might be related to many factors. Data from the light bar readings and leaf area measurements strongly suggest that the reduction in the light intercepted by the plant and the decrease in the functional leaf area might influence the yield reduction. In Russet Burbank, the increase of the light intercepted by the plant following 98 dap probably was a result of secondary shoot growth, a typical characteristic of this cultivar.

REFERENCES CITED

1. Beute, M. K., and Lockwood, J. L. 1968. Mechanism of increased root rot in virus infected peas. *Phytopathology* 58:1643-1651.
2. Close, R., 1964. Some effects of other viruses and of temperature on the multiplication of Potato Virus X. *Ann. Appl. Biol.* 53:151-164.
3. Damsteegt, V. D., and Ronde, M. R. 1993. Interactions between Maize Streak virus and Downy mildew fungi. *Plant Dis.* 77:390-392.
4. Davis, J. R., and Allen, T. C. 1984. Relationships of defined PVX infection levels to Verticillium wilt, yield, and quality of the Russet Burbank. *Am. Potato J.* 61:669-682.
5. De Bokx, J. A., and Huttinga, H. 1981. Potato virus Y. CMI/AAB Descriptions of Plant Viruses N.242. 6 pp.
6. De Bokx, J. A., and Piron, P. G. M. 1977. Effect of temperature on symptom expression and relative virus concentration in potato plants infected with potato virus Y^N and Y⁰. *Potato Res.* 20:207-213.
7. Goodell, J. J., Powelson, M. L., and Allen, T. C. 1982. Interrelations between Potato Virus X, Verticillium dahliae and Colletotrichum atramentarium in potato. *Phytopathology* 72:631-634.
8. Jones, R. A. C. 1990. Strain group specific and virus specific hypersensitivity reactions to infection with potyvirus in potato cultivars. *Ann. Appl. Biol.* 117:93-105.
9. Kogan, M., and Turnipseed, S. G. 1980. Soybean growth and assessment of damage by arthropods. *Sampling Methods in soybean Entomology* (M. Koga and D. c. Herzog, eds.). Springer-Verlag, New York. pp. 3-29.
10. Kotcon, J. B., Rouse, D. I., and Mitchell, J. E. 1985. Interaction of Verticillium dahliae, Collectotrichum coccodes, Rhizoctonia solani and Pratylenchus penetrans in early dying syndrome of Russet Burbank potatoes. *Phytopathology* 75:68-74.
11. Krikun, J., and Orion, D. 1979. Verticillium wilt of potato: importance and control. *Phytoparasitica* 7:107-

115.

12. MacGuidwin, A. E., and Rouse, D. I. 1990. Effect of Meloidogyne hapla, alone and in combination with subthreshold populations of Verticillium dahliae, on disease symptomatology and yield of potato. *Phytopathology* 80:482-486.
13. MacGuidwin, A. E., and Rouse, D. I. 1990. Role of Pratylenchus penetrans in the Potato Early Dying Disease of Russet Burbank potato. *Phytopathology* 80:1077-1082.
14. Martin, M. J., Riedel, R. M., and Rowe, R. C. 1982. Verticillium dahliae and Pratylenchus penetrans: Interactions in the early dying complex of potato in Ohio. *Phytopathology* 72:640-647.
15. Milliken, G. A., and Johnson, D. E. 1984. Analysis of messy data. Van Nostrand Reinhold Company. New York. p. 297-314.
16. Nicot, P. C., and Rouse, D. I. 1987. Precision and bias of three quantitative soil assays for Verticillium dahliae. *Phytopathology* 77:875-881.
17. Rahimian, M. K., and Mitchell, J. E. 1984. Relationship of Verticillium dahliae and Erwinia carotovora pv. carotovora in the early dying disease of potato. *Phytopathology* 74:327-330.
18. Rowe, R. C. 1983. Early dying - east and west. *Am. Veg. Grow.* 31(3):8-10.
19. Rowe, R. C., Davis, J. R., Powelson, M. L., and Rouse, D. I. 1987. Potato early dying: Causal agents and management strategies. *Plant Dis.* 71:482-489.
20. Rowe, R. C., Riedel, R. M., and Martin, M. J. 1985. Synergistic interactions between Verticillium dahliae and Pratylenchus penetrans in potato early dying disease. *Phytopathology* 75:412-418.
21. SAS Institute. 1988. SAS/STAT User's Guide. Release 6.03 edition. SAS Institute, Inc., Cary, NC. 1028 pp.
22. Sigvald, R. 1985. Mature-plant resistance of potato against Potato Virus Y⁰ (PVY⁰). *Potato Res.* 28:135-143.
23. Sutula, C. L., Gillet, J. M., Morrissey, S. M., and

- Ramsdell, D. C. 1986. Interpreting ELISA data and establishing the positive-negative threshold. Plant Dis. 70:722-726.
24. Yang, I., Reddick, B., and Slack, S. A. 1983. Results of experiments on the purification of potato virus Y. Phytopathology 73:794-797.

CHAPTER TWO

Effect of Timing of Potato Plant Infection by PVY
on Tuber Infection

INTRODUCTION

The common strain of Potato Virus Y , mainly spread by Myzus persicae (Sulz.), is among the most important potentially limiting factors for potato (Solanum tuberosum L.) production. It can cause rapid degeneration of seed-stocks. Recently, the incidence of PVY has been increasing in most of the potato growing areas in North America. In Wisconsin, a number of potato seed lots have been rejected by the seed certification agency due mainly to PVY infection. Reasons for the increase in incidence are not well understood; however, there are indications that it might be cultivar related (German, personal communication). A recently released cultivar, Russet Norkotah, is described as highly susceptible (7) and Russet Burbank is moderately susceptible to PVY (1).

Studies conducted by Beemster (3, 4) in Sweden showed that mature plant resistance to PVY, common strain, is cultivar dependent. Beemster (3) defined mature plant resistance as the acquired resistance of older potato plants to infection by PVY. Sivgald (9) found similar results and

showed that the older the potato plants are at the time of infection the less the virus will reach the tubers.

An experiment conducted in 1991 at Hancock, Wisconsin, (USA) indicated that early infection, about 30 days after planting, by PVY, resulted in about 60% of progeny tubers infected with the virus (not published).

The present study was initiated to investigate the effect of plant infection by PVY at different developmental stages on the infection of progeny tubers.

MATERIALS and METHODS

Plot establishment. This study was conducted in 1992 at the Hancock Research Station. The experiment was designed as a Randomized Block Design (RBD) with four replicates and four treatments. Treatments were randomly assigned to plots within each block. The experiment included the following treatments: mechanical inoculation with PVY at 30, 55 and 90 days after planting (dap) in Russet Norkotah, at 30, 55, 75 and 95 in Russet Burbank and a control.

Each experimental unit consisted of four rows. The two middle rows were used for sampling. The distance between the rows was 91 cm and the seed pieces were planted 30 cm apart in the row.

Mechanical inoculation of potato plants with PVY at different dates. Detached leaves from a single PVY infected plant, were collected and ground with Phosphate Buffer Solution (PBS) pH=7.4, in a proportion of 1:10 of plant tissue weight: buffer volume. The extract was filtered with cheese cloth and the resulting solution was gently rubbed with a paint brush over all the foliage of the plants already dusted with carborundum. The inoculum was chilled over ice in a cooler until it was applied to the leaves. The inoculum was applied only in the sampling area.

The remaining infected leaves were frozen and used in later inoculations. A sample of the inoculum used on each day of inoculation was kept and frozen. At the end of the season the frozen extracts were tested using ELISA and infectivity test.

After harvesting, ten tubers were randomly selected from each plot and assayed individually for PVY. This assay was done on the sprouts. The tubers were treated with Rindite to produce earlier and massive sprouts. Rindite is an organic solution containing ethylene chlorohydrin, dichloroethane and carbon tetrachloride in a proportion of 7:3:1 respectively. Four to five sprout tip pieces, approximately 0.05 grams of tissue, were cut from each tuber with a sterile scalpel. The sprout pieces were then placed immediately in a microcentrifuge tube containing 0.5 milliliters of enriched

sample extract buffer solution (Phosphate buffer solution, containing Tween 20, egg albumin, polyvinyl pyrrolidone and sodium sulfite), equivalent to a dilution ratio of about 1:10 of tissue weight and extract buffer volume. Then the tissue was ground in a 1.5 ml microtube with a disposable polypropylene pellet pestle mixer. A micropipet was used to collect 100 microliters of the sample which was analyzed by DAS-ELISA.

RESULTS

The results from the DAS-ELISA test on the progeny tubers indicated a trend toward a decrease over time of the number of progeny tubers infected with PVY in both cultivars (Fig. 1). Late plant infection resulted in a low percentage of progeny tubers infected by PVY. For example, tuber infection treatment resulted in about 80% and 70% of progeny tubers infected by PVY on Russet Norkotah and Russet Burbank, respectively; and if inoculation was done 90 days after planting, only 20% of progeny tubers became infected. The exception was observed in the mechanical inoculation 30 days after planting. Tubers harvested from that treatment showed a very low percentage of infection.

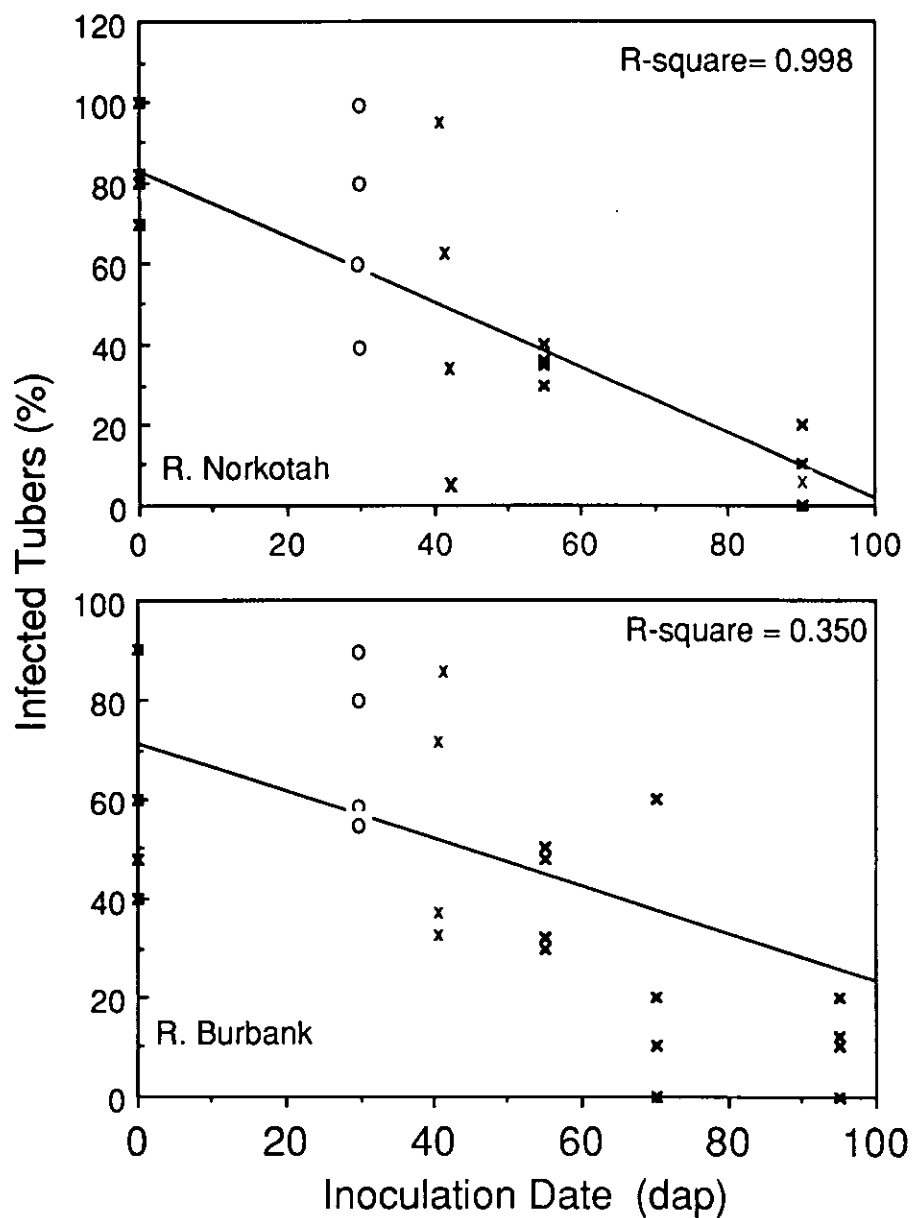


Figure 1: Effect of PVY inoculation at different dates on the infection of progeny tubers for Russet Norkotah and Russet Burbank. The symbol (o) represents data from 1991 and (x) represents data from 1992.

DISCUSSION

The results of this preliminary study are similar to those reported by Beemster (2) and Sivgald (9). In general, it was found that older plants are more resistant to virus translocation than younger ones. This phenomenon is called mature plant resistance (5). For one treatment, plant inoculation at 30 days after planting resulted in a low percentage of PVY-infected progeny tubers. That outcome suggests that plant infection by PVY might be influenced by environmental conditions. The growing season, in 1992, was mostly cool and frost occurred about four weeks after virus inoculation. Beemster (5) reported that the speed of the process of replication is affected by the environmental conditions, especially temperature. The effect of temperature on the expression of PVY symptoms was also studied by De Bokx and Piron (8). The results showed that low temperature reduced the virus titer and the expression of PVY symptoms on the foliage. Another study, conducted by Close (6) indicated that the optimum temperature for PVY multiplication was 28° C.

REFERENCES CITED

1. Bagnall, R. H., and Tai, G. C. C. 1986. Field resistance to potato virus Y in potato assessed by cluster analysis. Plant Dis. 70:301-304.
2. Beemster, A. B. R. 1965. Translocation of potato virus Y in potato plants after infection by means of aphids. Mededelingen Instituut voor Plantenziektenkunding Onderzoek 408:1786-1795.
3. Beemster, A. B. R. 1972. Virus translocation in potato plants and mature-plant resistance. In: J. A. De Bokx (Ed), Viruses of potatoes and seed potato production. p.144-151.
4. Beemster, A. B. R. 1976. Translocation of the potato viruses Y^N and Y⁰ in some potato varieties. Potato Res. 19:169-172.
5. Beemster, A. B. R. 1987. Virus translocation and mature-plant resistance in potato plants. Viruses of potatoes and seed potato production / edited by J. A. De Bokx and P. J. H. Van der Want. 2nd ed. Wageningen: Pudoc (Centre for Agricultural Publishing and documentation). p.116-125.
6. Close, R., 1964. Some effects of other viruses and of temperature on the multiplication of potato virus X. Ann. Appl. Biol. 53:151-164.
7. Gurwen, D., Weis, G. G. et al. 1991. Commercial vegetable production. A3422. University of Wisconsin Extension. p. 64-77.
8. De Bokx, J. A., and Piron, P. G. M. 1977. Effect of temperature on symptom expression and relative virus concentration in potato plants infected with potato virus Y^N and Y⁰. Potato Res. 20:207-213.
9. Sigvald, R. 1985. Mature-plant resistance of potato against Potato Virus Y⁰ (PVY⁰). Potato Res. 28:135-143.

CONCLUSIONS

The interactive effect between PVY and V. dahliae on the severity of PED and on potato yield was evaluated. In addition, a preliminary study of the effect of time of plant inoculation on the infection of progeny tubers was also conducted. The results of the study indicated the following:

1. In PVY infected tuber plots, symptom expression of PED was accelerated by about 10 days compared to the treatments with the fungus alone. Coinfection by the virus and the fungus increased the severity of PED. The effect of the interaction was synergistic.
2. The proportion of A size tubers (4-16 ounces) was reduced by each disease individually. The effect of both diseases present in the plots was additive.
3. Mechanical inoculation of PVY may cause yield losses of 20% and 30% on Russet Norkotah and Russet Burbank cultivars, respectively. The degree of yield loss may be influenced strongly by low temperature during the growing season. Planting seed with 60% tuber infection reduced yield to about 50% in both cultivars.

4. When potato plants were inoculated at different dates, it was found that the older the plants the less virus reached the progeny tubers. Based on previous studies conducted by Beesmter (2) and Sigvald (3) it suggests that mature-plant resistance is involved. PVY-infected tubers resulted in about 80% and 70% progeny tubers infected with PVY on Russet Norkotah and Russet Burbank, respectively, and the inoculation at 30 days after planting produced about 60% infected progeny tubers compared to about 20% if plants were inoculated 90 days after planting.

5. PVY infection by mechanical inoculation is cultivar related and it might be influenced by environmental conditions. The first inoculation performed 30 days after planting did not give any effect on symptom expression nor on potato yield or on infection of progeny tubers. 1992 was a cool season and besides that the temperatures about three weeks after inoculation were very low. The minimal and maximal temperature (average of 7 days) on the third week after inoculation was 6.4 and 18.8 degrees centigrade. According to Close (1) the optimal temperature for PVY multiplication is 28 °C.

Future directions for research on the PVY and V. dahliae should include effect of virus concentration on the severity

of PED, evaluation of the role of mature plant resistance on virus translocation, the effect of environmental conditions on plant infection by PVY (virus replication, virus translocation and symptom expression), and the mechanism for the synergistic interaction on symptom expression.

To the best of our knowledge, this is the first report on the effect of the interaction between PVY and V. dahliae in any plant. This information will provide a better understanding for control measures, including plant breeding and seed certification programs.

REFERENCES CITED

1. Close, R., 1964. Some effects of other viruses and of temperature on the multiplication of potato virus X. *Ann. Appl. Biol.* 53:151-164.
2. Beemster, A. B. R. 1972. Virus translocation in potato plants and mature-plant resistance. In: J. A. De Bokx (Ed), *Viruses of potatoes and seed potato production.* 144-151.
3. Sigvald, R. 1985. Mature-plant resistance of potato against Potato Virus Y⁰ (PVY⁰). *Potato Res.* 28:135-143.

APPENDIX I

Effect of Verticillium dahliae on PED in the presence
of PVY under controlled environmental conditions

INTRODUCTION

This study was conducted to investigate the relationship between PVY and V. dahliae on the severity of Potato Early Dying (PED) disease under controlled environment conditions. Potato Early Dying (PED), caused primarily by Verticillium dahliae, and Potato Virus Y are among the most important diseases in many potato growing areas (14).

Many studies have demonstrated that the severity of PED may be enhanced by other factors like other organisms, cultivar and environmental conditions (4, 7, 9, 12). Although there are very few studies that deal with interactions between Verticillium and a virus, there are a number of reports that demonstrated virus-fungus interactions (1, 6, 11, 16, 17). It has been reported that the effect of fungus-virus interactions varies according to whether the fungus is a facultative-saprophyte or obligate parasite. Studies have shown that double infection by a virus and a facultative saprophytic fungus usually result in a synergistic effect (1, 6, 11, 16), but if the fungus is an obligate parasite then the reaction is protective (17). Interaction between PVY and Verticillium

dahliae has not been reported.

In 1991 a field study was conducted to determine the effect of the interaction between PVY and V. dahliae. The trial was established at Hancock, Wisconsin (chapter one). To evaluate the effect of the interaction under controlled environment, a greenhouse experiment was performed in spring 1992.

The objectives of this research were to determine how vascular colonization by V. dahliae is affected in the presence of PVY and to determine if double infection by PVY and V. dahliae accelerates the symptom expression of PED.

MATERIALS AND METHODS

The experiment was conducted in March - May 1992 at the greenhouse, UW-Madison.

Seed source. The cultivar Russet Norkotah was used. Russet Norkotah is highly susceptible to Vd and moderately susceptible to PVY. Tubers harvested in 1991, from healthy and PVY-infected potatoes, were used as seed.

Before planting, the tubers were tested for PVY. Virus-infected seeds were used in specific tuber infected treatments.

Experimental design. The experiment used a completely randomized design with five replicates and nine treatments.

The experiment included the following treatments: PVY foliar infection, fungus in low concentration, fungus in high concentration, PVY tuber infection, combinations of PVY foliar infection with the fungus in low concentration, combinations of PVY foliar infection with the fungus in high concentration, the same combinations for PVY tuber infection, and a control. For low and high concentrations of the fungus 1 and 10 g of inoculum were applied to the soil. The fungus in high concentration was equivalent to its threshold level.

Each experimental unit consisted of two pots filled with potting mix soil. The soil was prepared by combining 4 cubic feet of sphagnum peat, 6 cubic feet of torpedo sand and 500 g of calcium carbonate lime.

Tubers were cut by hand into quarters, leaving each seed piece with about 3 eyes. One day before planting the soil contained in pre-selected pots was infested with the fungus. Planting was done on March 13 and two tubers were planted in each plot at a depth of 7.5 cm. Thirty days after planting selected plants were mechanically inoculated with the virus. Plants were sprayed with wide spectrum insecticides, Avid and Talstar, to control whiteflies. Plants were watered about twice per week.

Vd Inoculum and Soil Infestation. The inoculum of V. dahliae was produced by growing the fungus on soaked and sterile rye

seed, contained in a mason jar at room temperature and in dark conditions. After 4-6 weeks of incubation the rye seeds were air dried and ground in a Wiley mill.

The inoculum of the fungus was mixed with the soil in a 5 gallon bucket. The inoculum was prepared according to the protocol described by Nicot and Rouse (10). The inoculum concentration was expressed in colony forming units (cfu) per gram of soil.

PVY inoculum and PVY mechanical inoculation. Detached leaves from an infected plant were ground with Phosphate Buffer Solution (PBS) pH=7.4, in a proportion of 1:10 of plant tissue weight: buffer volume. The extract was filtered with cheese cloth and the resulting solution was gently rubbed over the foliage of selected plants. Four complete leaves were randomly selected at different positions on the plant.

Disease severity. The amount of disease was measured in terms of disease severity over time. Also, assays for PVY as well as for V. dahliae were conducted. The soil and plant sap was assayed for the fungus and the foliage for the virus.

Soil Assay. Soil samples were taken at harvest from each of the individual pots and assayed for V. dahliae using the soil-dilution plating technique described by Nicot and Rouse (10).

The estimate of soil inoculum in a sample was expressed as an average number of colony-forming units (cfu) of V. dahliae per gram of dry soil.

Plant Assay For Vd. To evaluate the magnitude of the colonization by the fungus, stems were collected at 30, 40, 55 and 80 days after planting (dap). The sap was extracted and plated according to the procedure described in chapter one (materials and methods).

Plant Assay for PVY on the Foliage. Leaflets collected 55 and 80 dap were tested for PVY by using the DAS-ELISA test, following the procedure provided by AGDIA Inc. The interpretation of the positive-negative threshold was done according to Sutula (15).

Sequential rating of disease severity. Plants were visually scored for disease symptoms at different dates. Disease symptoms were assessed based on the scale described in chapter one; however, plants were evaluated individually.

RESULTS AND DISCUSSION

Disease assessment. Symptoms of PED, as well as viral symptoms, from infected tubers, were evident about 30 days after planting. Early symptoms of PED were characterized by

yellowing of the lower leaves and plants infected by PVY had mosaic symptoms on the second leaf. At this time, virus symptoms were also observed in some plants that were not grown from infected tubers. Although the tubers were tested for PVY before planting, the assumption is that some tubers used as seed were contaminated by the virus. Probably the infected tubers were not detected because the ELISA test was not conducted on all the sprouts from each tuber. Tubers used in this experiment were not treated with Rindite because they were already sprouted. On the other hand, most of PVY treatments did not show viral symptoms, especially the foliar infection treatments. The disease assessment over time is represented in table I-1.

Assay for PVY on the foliage. Although viral symptoms were not observed in many plants, the ELISA test showed that most of the plants were infected by PVY, including the control treatment. Many factors may be considered for these results; among them are: late infection of the plants in the field by aphids or mixing of treatments at harvesting and grading. The results are shown in table I-2.

Vascular colonization by the fungus. The results of the sap assay for V. dahliae showed no differences among treatments due to the presence of PVY. However, the two levels of the

Table I-1: Disease severity average for PVY and PED in a scale of 0-4 (0 = no symptoms, 1 = < 25% PVY or PED symptoms, 2 = 25- 50%, 3 = > 50%, but not dead plant and 4 = dead plant).

<u>Inoculum</u>	<u>PED Severity</u> (Days After Planting)				<u>PVY Severity</u> (Days After Planting)			
	<u>30</u>	<u>40</u>	<u>55</u>	<u>65</u>	<u>30</u>	<u>40</u>	<u>55</u>	<u>65</u>
Control	0.1	0.5	0.0	0.2	0.0	0.0	0.7	1.5
TbPVY	0.0	1.3	0.4	0.8	0.0	0.0	0.0	1.4
FPVY	0.0	1.0	0.7	1.3	0.0	0.0	0.0	0.7
Vd(Low)	0.0	0.6	1.5	2.0	0.4	0.0	0.6	0.0
Vd(High)	0.2	1.4	2.3	3.0	0.4	0.0	0.0	*
TbPVY+VL	0.0	0.8	1.1	1.4	0.0	0.0	1.2	0.4
TbPVY+VH	0.2	1.7	2.4	3.0	0.0	0.0	0.9	*
FPVY+VL	0.1	0.6	0.6	0.7	0.0	0.0	0.0	0.0
FPVY+VH	0.4	1.8	3.1	3.6	0.0	0.0	*	*

The abbreviations and symbols have the following meaning:

TbPVY - Tuber infection

FPVY - Foliar infection

Vd(Low) or VL- Fungus in low concentration

Vd(High) or VH-Fungus in high concentration

(*) Missing data.

Table I-2: Relative Virus Content on the Foliage.
(Positive-negative threshold = 0.1)

<u>Optical Density (490 nm)</u>								
<u>Inoculum</u>	<u>55 dap</u>				<u>80 dap</u>			
	<u>I</u>	<u>II</u>	<u>III</u>	<u>IV</u>	<u>I</u>	<u>II</u>	<u>III</u>	<u>IV</u>
Control	0.151	0.090	0.347	0.523	0.605	0.038	0.051	0.084
TbPVY	0.576	0.168	0.534	0.359	0.338	0.611	0.098	1.050
FPVY	0.379	0.088	0.478	0.092	0.070	0.465	1.136	0.334
Vd(Low)	0.160	0.057	0.067	0.312	0.008	0.084	0.040	0.021
Vd(High)	0.041	0.095	0.058	0.101	0.125	0.115	0.119	0.102
TbPVY+VL	0.817	1.057	0.139	0.527	0.806	0.256	1.474	0.184
TbPVY+VH	1.515	0.476	0.904	0.139	0.738	0.454	0.540	0.721
FPVY+VL	0.213	0.076	0.074	0.181	0.061	0.075	0.155	0.060
FPVY+VH	0.460	0.046	0.053	0.084	0.038	0.065	0.085	0.042

The abbreviations have the following meaning:

TbPVY - Tuber infection

FPVY - Foliar infection

Vd(Low) or VL- Fungus in low concentration

Vd(High) or VH-Fungus in high concentration

fungus seem to be significantly different (Table I-3).

Inoculum density. The amount of propagules recovered from soil in different pots within the same treatment was highly variable. However, it was observed that in pots with virus-infected plants, the number of propagules recovered from soil tended to be higher if compared to those with the fungus alone. The increase in the amount of fungus in the soil was evident in treatments with a combination of both pathogens. For example, about 40 cfu/g of soil were recovered in treatments with PVY infected tubers with the fungus in high concentration, compared to 10 cfu/g of soil for the fungus alone. Like wise, but in lower amounts, treatments with the combination of foliar infection by PVY and the fungus in high concentration resulted in about 20 ppg. No differences were observed in treatments with the fungus in low concentration. Although these results are highly variable, they suggest that the presence of the virus might have an effect on the multiplication of the fungus in the soil. Beute and Lockwood (2) reported that virus-infected plants increased exudation of many kinds of compounds, including nutrients utilizable by fungi. Dammsteegt (3) also reported that a possible mechanism of the susceptibility of plants infected with virus to diseases caused by soil-borne fungi may include acceleration of spore germination and alteration of the host metabolism.

Table I-3: Stem colonization by the fungus 80 dap.Colony Forming Unit / microliter of sap

<u>Inoculum</u>	<u>I</u>	<u>II</u>	<u>III</u>	<u>IV</u>	<u>V (Replicates)</u>
Control	0	0	0	0	0
TbPVY	0	0	0	0	0
FPVY	0	0	0	0	0
Vd(Low)	0	61	0	0	0
Vd(High)	661	428	19	24	*
TbPVY+VL	12	15	0	30	*
TbPVY+VH	1	8	311	330	*
FPVY+VL	0	5	2	8	4
FPVY+VH	93	16	562	7	169

The abbreviations and symbols have the following meaning:

TbPVY - Tuber infection

FPVY - Foliar infection

Vd(Low) or VL- Fungus in low concentration

Vd(High) or VH-Fungus in high concentration

(*) No data.

Any conclusion from this trial is preliminary. The results were highly variable. The causes for the variability are not known.

To conduct such a study it is important to consider the seed source to be used. From the results of the field experiments conducted in 1991 and 1992 it seems that it is more appropriate to use foundation seed.

REFERENCES CITED

1. Bateman, D. F. 1961. Synergism between Cucumber Mosaic Virus and Rhizoctonia in relation to Rhizoctonia damping-off of cucumber. *Phytopathology* 51:574-575 (Abstr.).
2. Beute, M. K., and Lockwood, J. L. 1968. Mechanism of increased root rot in virus infected peas. *Phytopathology* 58:1643-1651.
3. Damsteegt, V. D., and Ronde, M. R. 1993. Interactions between Maize Streak virus and Downy mildew fungi. *Plant Dis.* 77:390-392.
4. Davis, J. R., and Allen, T. C. 1984. Relationships of defined PVX infection levels to Verticillium wilt, yield, and quality of the Russet Burbank. *Am. Potato J.* 61:669-682.
5. De Bokx, J. A., and Huttinga, H. 1981. Potato virus Y. CMI/AAB Descriptions of Plant Viruses N.242. 6 pp.
6. Farley, J. D., and Lockwood, J. L. 1964. Increased susceptibility to root-rots in virus-infected peas. *Phytopathology* 54:1279-1280.
7. Goodell, J. J., Powelson, M. L., and Allen, T. C. 1982. Interrelations between Potato Virus X, Verticillium dahliae and Colletotrichum atramentarium in potato. *Phytopathology* 72:631-634.
8. Krikun, J., and Orion, D. 1979. Verticillium wilt of potato: importance and control. *Phytoparasitica* 7:107-115.
9. MacGuidwin, A. E., and Rouse, D. I. 1990. Role of Pratylenchus penetrans in the Potato Early Dying Disease of Russet Burbank potato. *Phytopathology* 80:1077-1082.
10. Nicot, P. C., and Rouse, D. I. 1987. Precision and bias of three quantitative soil assays for Verticillium dahliae. *Phytopathology* 77:875-881.
11. Nitzany, F. E. 1966. Synergism between Pythium ultimum and Cucumber Mosaic Virus. *Phytopathology* 56:1386-1389.
12. Rowe, R. C., Davis, J. R., Powelson, M. L., and Rouse, D. I. 1987. Potato early dying: Causal agents and

management strategies. Plant Dis. 71:482-489.

13. Schroth, M. N., and Teakle, S. D. 1963. Inference of virus and fungus lesions on plant exudation and chlamydospore germination of Fusarium solani f. sp. phaseoli. Phytopathology 53:610-612.
14. Slack, S. A. 1992. A look at Potato Leafroll Virus and PVY: past, present and future. Valley Potato Grower 35-39.
15. Sutula, C. L., Gillet, J. M., Morrissey, S. M., and Ramsdell, D. C. 1986. Interpreting ELISA data and establishing the positive-negative threshold. Plant Dis. 70:722-726.
16. Thanassouloupoulos, C. C. 1976. Symptom expression of tomato wilt fungi Verticillium and Fusarium as affected by the presence of Tobacco Mosaic Virus. Phytoparasitica 4:137-140.
17. Wilson, E. M. 1958. Rust-TMV cross protection and necrotic-ring reaction in bean. Phytopathology 48:228-231.

APPENDIX II

Table II-1: Average number of Vd Propagules recovered from soil (cfu/g of soil).

Cultivar	Inoculum	1991		1992	
		Mean	s	Mean	s
R. Norkotah	Check	0	-	0	-
	Vd (Low)	8	1	13	9
	Vd (High)	0	-	17	12
R. Burbank	Check	0	-	0	-
	Vd (Low)	0	-	22	20
	Vd (High)	9	2	35	19

Symbol "s" represents the standard deviation.

Table II-2: Percentage of progeny tubers infected by PVY in 1992.

Inoculum	<u>Cultivar</u>	
	R. Norkotah	R. Burbank
Check	0	0
TbPVY	82.5	38.0
TbPVY + Vd (Low)	78.0	16.0
TbPVY + Vd (High)	72.0	36.0

APPENDIX III

PVY characterization

Absorption spectrum for PVY. The purified preparation was scanned from 320 to 220 nm in a spectrophotometer, using a dilution of 1:100. The absorption spectrum for the purified preparation is represented in figure III-1.

The ratio of the absorbance at 260 nm with the absorbance at 280 nm is equal to 1.22.

PVY Morphology. The purified preparation of PVY was analyzed by negative-staining electron microscopy. The micrograph is represented in figure III-2.

PVY concentration. The determination of the virus concentration was based on optical density of the purified preparation at 260nm and the extinction coefficient (E) for PVY.

$$E = OD_{260} (1\text{mg/ml, } 1 \text{ cm light path}) = 2.9;$$

$$OD_{260} (\text{purified preparation}) = 0.209, \text{ using a dilution of } 0.1\%$$

$$\text{Purified PVY concentration} = 7.21\text{mg/ml}$$

PVY yield. From 470 g of plant tissue used for the PVY purification 2.280ml of resuspended virus was obtained.

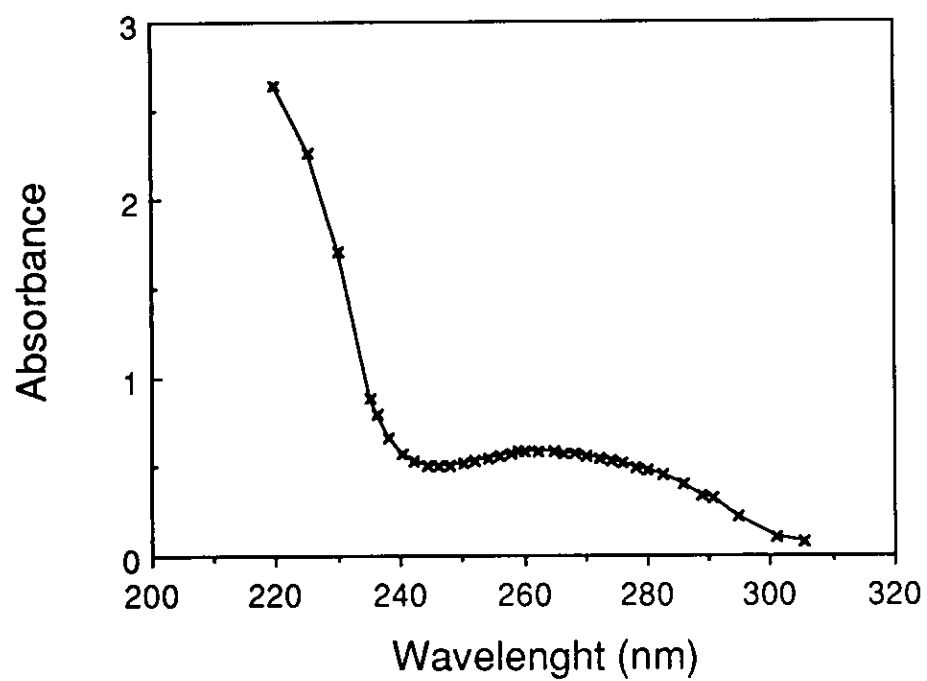


Figure III-1: PVY absorption spectrum.



Figure III-2: Electron micrograph of PVY.

The yield of PVY preparation was calculated based on the virus concentration and the volume of the virus preparation.

PVY Yield preparation = 35.0 mg/Kg of plant tissue

Negative-positive threshold. The calculation of the negative-positive threshold was based on the mean value of the negative control (m) and its standard deviation (s). A positive result was the one with its optical density value equal or higher than the sum of $(m + 4s)$.

$$m = 0.0522$$

$$s = 0.0198$$

$$m + 4s = 0.13$$

Infectivity test. The infectiveness of the inoculum used in the field was evaluated based on the number of local lesions produced on the indicator plant, Chenopodium amaranticolor (Fig.III-3). The indicator plant was mechanically inoculated with PVY inoculum. The results are represented in Table III-1.

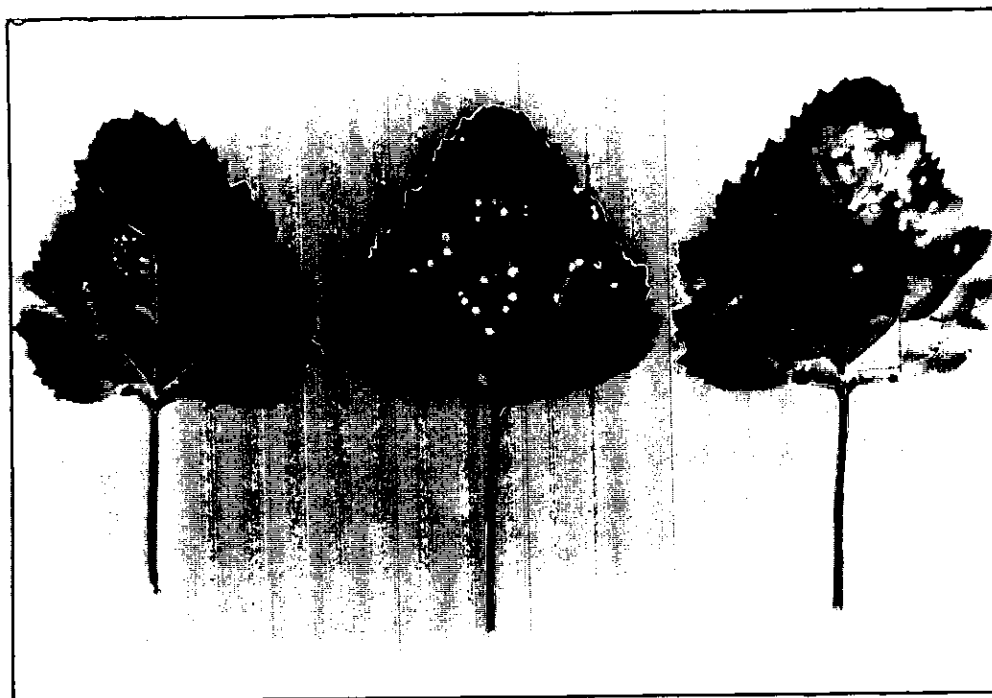


Figure III-3: Local lesions caused by PVY on *C. amaranticolor*.

Table III-1: Average number of local lesions on *C. amaranticolor*

Inoculum level	Plant #	<u>Days after inoculation</u>			
		3	6	9	12
Check	1	0*	0	0	0
	2	0	0	0	0
	3	0	0	0	0
1:10	1	0	1	3	3
	2	0	1	2	3
	3	0	12	14	16
1:100	1	0	0	0	0
	2	0	3	5	5
	3	0	0	0	0

(*) Average number of lesions from four leaves per plant.