The effect of weeds on the value of rotation as a practical control for Verticillium wilt of potato

***L.V. Busch*[1], *Elizabeth A. Smith*[1], *and Fritz Njoh-Elango*[2]**

In microplot tests in Ontario rotation of potatoes with barley or fallow proved ineffective in controlling Verticillium wilt caused by ***Verticillium dahliae*** or ***Verticillium albo-atrum.*** Several weed species were excellent hosts of ***V. dahliae,*** and microsclerotia were produced on their roots within 2 weeks of inoculation. In soil samples averaging 1.3 microsclerotia of ***V. dahliae*** per g of soil 80-90% of the Kennebec potato test plants became infected. It is suggested that variations in the number of viable microsclerotia in soil at the start of a rotation experiment, the effectiveness of the weed control program, and the weather during the experiment could account for the controversy surrounding the value of rotation as a means of control. The authors suggest that in Ontario rotation would be of no value in controlling Verticillium wilt of potato.

Can. Plant Dis. Surv. 58:3, 61-64. 1978

Au cours d'essais en micro-parcelles effectues en Ontario, l'alternance de la sole de pomme de terre avec l'orge ou avec une jachère s'est révélée impuissante dans la lutte contre la fletrissure verticillienne causée par ***Verticillium dahliae*** ou ***Verticillium albo-atrum.*** Plusieurs espèces de mauvaises herbes constituaient d'excellents hôtes de ***V. dahliae*** et leurs racines produisaient des microslérotes moins de deux semaines apres l'inoculation. Dans les échantillons de sols possedant en moyenne 1.3 microsclerotes de ***V. dahliae*** par gramme de sol, 80 a 90% des plants d'essai de pommes de terre de la variété Kennebec ont été atteints. Il semblerait que des variations du nombre de microsclerotes dans le sol au debut de l'experience de rotation, l'efficacité du programme de désherbage et les conditions météorologiques prévalaient au cours de l'expérience pourraient partiellement expliquer la polémique sur la valeur de la rotation comme moyen de lutte. Les auteurs estiment qu'en Ontario, la rotation n'aurait aucune valeur pour la lutte contre la fletrissure verticillienne de la pomme de terre.

In Ontario Verticillium wilt caused by ***Verticillium albo-atrum*** Reinke & Berth and ***Verticillium dahliae*** Kleb. is a serious disease of potato, tomato, eggplant, strawberry, maple, and other hosts. The two pathogens are soil-borne fungi that attack their hosts through the root system. Another important source of inoculum is infested potato seed pieces. The latter may be the major recurring source of inoculum of ***V. albo-atrum,*** particularly in areas where potatoes are rotated with non-hosts such as grains or corn (13).

Verticillium wilt is difficult to control; resistant varieties, crop rotation, and soil fumigation with high-value crops are the three most commonly recommended practices. Currently there is no variety of potato recommended for Ontario which can be considered resistant and, until very recently, fumigation was considered uneconomical. This situation has changed in the past few years and fumigation is currently being seriously considered. However, because of our short growing season, fumigation must be done in the fall following harvest and in many years this may not be practical.

There is considerable controversy in the literature regarding the usefulness of rotation as a means of control. Results have been conflicting and in many cases difficult to explain (11, 12, 16, 18). Some of these difficulties may be due to the presence of broadleafed weeds in the treatment areas (13). Brown and Wiles **(2)** state "Adequate control of the weed hosts would also be required where crop rotation was used to reduce the inoculum potential of the fungus." Unfortunately in most cases with weed hosts there are no observable symptoms, with the possible exception of stunting, to indicate infection (18).

The effectiveness of rotation is also affected by the number of viable microsclerotia present in the soil and by the number of microsclerotia per gram of soil required for infection of a particular crop. Reports of these numbers vary widely, from 0.3 to 3000 per g of soil, depending upon the crop, the workers involved, and the method of estimating the number of microsclerotia present (1, 6, 8).

This paper reports results of a crop rotation experiment on the control of Verticillium wilt of potato caused by ***V. albo-atrum*** and ***V. dahliae,*** of studies on the number of microsclerotia of ***V. dahliae*** per gram of soil required for infection of potato plants, and on the effects weed hosts may have on the survival of ***V. dahliae.***

[1] ***Department of Environmental Biology, University of Guelph, Guelph, Ontario N1G 2W1.***

[2] ***Department of Plant Pathology, Macdonald College, Ste. Anne de Bellevue, Quebec.***

Accepted for publication on March 9, 1978

Materials and methods

Crop rotation

Potatoes were grown in the field in a range of 24 microplots constructed of reinforced concrete. Each plot was 1.2 x 1.2 x 0.8 m deep, sunk in the ground with the tops 10 cm above the soil surface to prevent soil water from washing into the plots from surrounding areas or adjacent plots. The plots were filled with sandy loam to within 10 cm of the top. Nine Kennebec potato seed pieces inoculated by dipping them into a water suspension of spores and mucelium of *V. dahliae* or *V. albo-atrum* were planted in each of the plots the first year. The potato tops were left on the surface of the soil over winter and were incorporated into their respective plots the following spring. After the first year, the potato seed pieces were washed prior to planting but were not inoculated. The following rotations were utilized: 1) one year fallow followed by two years potatoes, 2) one year barley followed by two years potatoes, 3) two years barley followed by one year potatoes, and 4) potatoes three years. A record was kept each year of the percent wilt present whenever a susceptible crop was grown. There were three randomized replications for each treatment. Sections from near the soil surface of each potato stem taken in early August were placed on moist filter paper in petri dishes and examined for conidiophores 1 week later (14).

Number of microsclerotia of V. dahliae required for infection of potato

Little information is available on the number of microsclerotia (MS) of *V. dahliae* per gram of soil necessary for infection and for subsequent development of symptoms in potato. To obtain microsclerotia for soil infestation studies, *Verticillium dahliae* was cultivated at 25°C in the dark for 4 weeks on a glucose-KNO, mineral salts medium (9) modified by the addition of 5 μg/litre biotin for maximum microsclerotia production.

Microsclerotia free from mycelium and conidia were obtained by using a modification of Congly's method (4, 5). The number of microsclerotia used to infest the soil was determined by weight. Two, 5, *8*, 10, 20, 40, 70 and 100 microsclerotia were mixed with a small amount of soil in a beaker and then added to a weighed air-dried sterile potting soil sample and mixed thoroughly. For every experiment, a representative soil sample was collected from each treatment mixture prior to planting to determine by three sampling techniques the number of viable MS present in the soil. The potato plants were grown under short day conditions (10 h light - 14 h dark) in environmental chambers at 22 ± 2°C. Light intensity at plant height was 16,000 lux provided by fluorescent and incandescent lamps (3). The plants were examined for symptoms 60 days after planting. The three soil isolation techniques used were: a) *Datura* root bioassay (7), b) soil washing (7), and c) soil dilution (15). Method B was modified by using a soil sample size of 1.0 g rather than 0.1 g as used by Evans (7). Method C was modified by reducing the quantity of agar in the medium by 6.3 g/litre to prevent gelling at 40-42°C; 1.0 g of sieved (1.0 mm) soil sample was added to 400 ml of cooled (40°C) agar, shaken to disperse the soil, and then poured into 20 plates. The soil sample was not diluted with water prior to use. Method C proved to be most reliable for the recovery of MS added to the **soul** but even it was quite variable.

Effect of weeds on Verticillium dahliae survival

Because of the erratic results obtained from the crop rotation experiments, various weeds were examined as potential hosts of *Verticillium.* In several experiments the weeds were grown in pots in the greenhouse or light chamber and inoculated by pouring over the **soil a** spore-mycelial suspension of *V. dahliae*. Complete plants were harvested weekly for 3 weeks starting **2** weeks after inoculation. The roots were washed and 100 cm of the smaller roots placed on water agar and examined for microsclerotia 1 week later (6). Representative cross sections of the stems and petioles were placed on moistened filter paper in petri dishes. These sections were examined periodically with the dissecting microscope for conidiophores of *Verticillium* (14).

Results

Rotation

Table 1 summarizes the results obtained from the various cropping sequences used during 1970-73. The results are difficult to explain and do not follow any readily apparent pattern. There is an indication that *V. dahliae* survives better, i.e. maintains a higher inoculum level in the absence of potatoes than *V. albo-atrum* but it is obvious from the data that other complicating factors were operating throughout the experiment. In 1974 eggplant *(Solanum melongena* L.) was planted in all of the plots and was 100% infected by mid August. It is also interesting to note that stunting of eggplant occurred only in the *V. dahliae* plots, possibly indicating that the plants were infected and colonized sooner than those grown in the *V, albo-atrum* plots.

Weed hosts

Table 2 indicates our results from many different tests over a 2-year period. In all of the weed species inoculated with *V. dahliae* the pathogen was isolated more often from the cortical tissue of the roots than from aboveground parts. Frequently microsclerotia were produced in the root tissue harvested at the first sampling date 2 weeks after inoculation; with some of the weed hosts the fungus was isolated only from the roots, never from the above ground parts. With the exception of groundsel *(Senecio vulgaris* L.), the weed hosts generally did not exhibit symptoms. Several cruciferous weeds proved to be infected and colonized by *V. dahliae.*

Table 1. Effect of cropping practices on survival of 2 species of ***Verticillium*** in a 4-year rotation in microplots

Crop sequence*	%potato plants infected†							
	Verticillium dahliae				***Verticillium albo-atrum***			
	1970	1971	1972	1973	1970	1971	1972	1973
1	100		40	70	100		6	49
2	100		55	56	100		>1	33
3	100			79	100			63
4	100	73	53	97	100	37	31	53

* 1 - Potatoes, fallow, potatoes, potatoes
* 2 - Potatoes, barley, potatoes, potatoes
* 3 - Potatoes, barley, barley, potatoes
* 4 - Potatoes each year
†Figures are means of 3 replications of 9 Kennebec potato plants/rep. The microplots were infested in 1970 with inoculated seed pieces of Kennebec potato; plant remains were incorporated into the soil each year. Infection was determined by isolation.

Table 2. Recovery of ***Verticillium dahliae*** from weed hosts grown in infested soil in pot tests

Weed host	No. of plants affected			No. of plants tested
	Root	Stem	Petiole	
Senecio vulgaris **L.**	19	11	11	20
Chenopodium album L.	29	22	17	29
Thlaspi arvense **L.**	18	8	7	18
Amaranthus retroflexus **L.**	22	5	5	22
Solanum nigrum L.	6	1	6	20
Malva pusilla **Sm.**	2	3	7	22
Malva neglecta Wallr.	2	0	2	16
Capsella bursa-pastoris **(L.)** Medic.	3	0	0	14
Cichorium intybus L.	1	0	0	2
Portulaca oleracea **L.**	18	12	sessile	27
Medicago lupulina L.	12	1	0	23

Table 3. Relationship between **soil inoculum** density and Verticillium wilt index of potatoes grown in microplots in 1975

Plot no	Inoculum density (no colonies/ 100 cm *Datura* root)	Inoculum density (MS/g soil)	Wilt index†
85	7	5 35	1 8 8
86	7	5 35	2 00
87	1	1 2 7	2 38
88	3	2 63	2 13
89	1	1 2 7	2 14
90	8	**6** 03	2 13
91	1	1 2 7	1 8 3
92	**8**	6 03	2 75
93	1	1 2 7	2 13
94	6	4 67	2 75
95	2	1 9 5	2 13
96	23	**16** 23	2 88

Converted according to Evans regression equation (Y = 0 59 + 0 68X)
† Wilt index on a 1-5 scale, 1 = no symptoms, 5 = plant dead

Number of microsclerotia required for infection

The threshold level of infection (defined as the minimum number of MS recovered per gram **of** soil in which potatoes became infected) was 1 MS/g soil; at this level the wilt index averaged 1.54 on a scale of 1 to 5 (1 - no symptoms, 5 - plant dead). Isolations from the microplots in 1975 also indicated a threshold level of infection of approximately 1 MS/g, with wilt indices ranging from 1.83 to 2.38 (Table 3).

Discussion

Rotation has been proposed **for** many years as a control for Verticillium wilt. The results have been variable, generally disappointing, and usually unpredictable. Many of the rotation experiments were done under circumstances in which even with the best of care some mixing of the soil from one plot to another would be expected. While this was impossible under our set up, our results would still indicate that rotation would be ineffective as a means **of** control.

We suspected that part of the problem of variability in our rotation study could be traced to inadequate weed control. While every effort was made to eliminate weeds by hand, some were missed or grew for 2-3 weeks before removal. At that time, we did not appreciate the fact that microsclerotia could be produced on the roots of 2-week-old seedlings. When these weeds were pulled many of the smaller roots would be left in the soil and these could have produced sufficient microsclerotia to account for the variability encountered.

In *V. dahliae,* microsclerotia are the principal, if not the only, propagules that persist in the soil and ensure the survival of this fungus. The microsclerotia develop in infected moribund potato or other host tissue, including weeds, above or below ground and are returned to the soil when this tissue is incorporated in the normal tillage procedures (5, 10, 17). The fact that the wilt index did not increase at the same rate as the number of viable MS recovered (Table 3) suggests that 1 MS/g soil would be sufficient to infect almost 100% of the potato crop with almost the same wilt index as from much larger numbers, e.g. **16,** of MS/g **soil.** The percentage of plants infected varied from 84 to 92 in these plots in 1975 and the previous crop history or the previous percentage of plants infected did not appear to have any effect on this.

The variability in the percent infection encountered from year to year in treatment 4 (Table 1) (potatoes each year) suggests that the number of viable microsclerotia necessary to achieve any given percentage of infection varies from one year to the next. Huisman and Ashworth (10) noted a reduction in the MS in cotton soils which was independent of the crop grown and they suggest that soil temperature-moisture interactions could be involved. The fact that the environment affects not only the expression of symptoms but **also** the survival **of** the microsclerotia and probably the actual infection of the plant itself must be considered when attempting to correlate number of microsclerotia per gram of soil, percentage of plants infected and severity of symptoms produced. It may very well be that in experiments where rotation gave control the actual number of MS/g soil was low or larger numbers of MS were required for infection and symptoms to be expressed under the particular condition existing at the time. Rotation under these circumstances could well lower the number of MS/g soil down to a point where the effect could be measured on the percentage of plants infected in subsequent crops.

While Verticillium has not been reported from cultivated crucifers in Ontario our results Table 2; penny cress (***Thlaspi arvense* L.)** and shepherd's purse ***(Capsella bursa-pastoris)*** (L.) Medic. suggest that infection of field grown crucifers is a distinct possibility. In addition, field collected ***Sisymbrium officinale* (L.)** Scop (hedge mustard) from potato soils yielded cultures of ***V. dahliae*** although the plants were showing no symptoms.

It is the authors' opinion that with MS numbers running as high as 78/g soil in our major potato growing area in Ontario (unpublished results) and with the prevalence of weed hosts in the alternate crops grown, rotation may be valuable as an agronomic practice, but it will do little or nothing to control Verticillium wilt. The particular crop involved in the rotation (as long as it is a non-host) is not as important as the weed control which to be effective must be as close to 100% as possible.

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