Transmission of *Verticillium dahliae* in Potato Tubers

A. Nachmias and J. Krikun

Agricultural Research Organization, Gilat Experiment Station, Mobile Post haNegev 2, Israel.
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ABSTRACT

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Studies were undertaken to establish if a relationship exists between foliar symptoms and tuber infection caused in potato by *Verticillium dahliae*. Examination of a large number of cultivars showed that there was no correlation between foliar symptoms and tuber infection or yield. Furthermore, year-to-year fluctuations of tuber infection were large. A proposal is made that potato seed certification be based on serological tuber examination and not on the foliar symptoms incited by the pathogen.

Additional key words: potato seed certification, seedborne pathogens, *Solanum tuberosum*.

The soilborne pathogen *Verticillium dahliae* Kleb. has a wide distribution and an extensive host range. In Israel, some 24 crop hosts and numerous weed hosts are known (3). A factor that leads to the rapid buildup of inoculum density of this fungus in potatoes is the mass production of microsclerotia on haulms of the autumn-sown crop (2). Potato crops are used in most of our rotations; *V. dahliae* became one of the main constraints on production of a number of crops (3).

The role of surface- and internally borne inoculum of seed-potato tubers in disseminating the pathogen has been pointed out by several workers (1,2,4,6,7,9). Infection by *V. dahliae* is a factor in potato seed certification programs in both Israel and the USA (8).

In Israel, there are two main potato-growing seasons: spring-sown during February, and autumn-sown during August. Seed tubers imported from Europe are used for the spring planting and a portion of the yield obtained from that crop is used as seed for the autumn planting. When the susceptible cultivar Up-to-Date was grown, symptoms of the disease were readily visible, and fields with ⩾5% symptom expression were rejected for certification. However, with the adoption of tolerant cultivars such as Desiree, Alpha, and Spunta, it became difficult to assess disease symptoms visually.

The purpose of the research reported here was to examine: the correlation between visible haulm symptoms and daughter-tuber vascular infestation caused by *V. dahliae*; the effect of sowing infected seed tubers on subsequent haulm infection; the effect of growing season conditions on tuber infection; and the correlation between symptom expression, total yield, yield of marketable tubers, and percentage of tuber infection in a representative number of cultivars.

MATERIALS AND METHODS

Relations between foliar symptoms, percent isolation of *V. dahliae* from haulms, and tuber infection. During the period from 1980 to 1982, samples of seed potato stocks were obtained from eight commercial fields. *V. dahliae* infection in the plants was determined by observing visible symptoms (eg—chlorosis, necrosis of leaves, and veinal discoloration) or by isolation of the pathogen

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from stems sampled from 100 randomly selected hills. Infection in the seed tubers obtained from these fields were determined by isolation of *V. dahliae* from the xylem vessels. The sample size ranged from 120 to 880 tubers, depending on the size of the field; individual tuber weights were 60–90 g. In addition, 1,000 tubers of each lot were kept in storage at 4 C and sown in the autumn in fumigated plots after careful surface disinfestation by dipping in a solution containing methoxyethylmercury chloride (0.03% a.i.) and pentachloronitrobenzene (0.75% a.i.) for 5 min. The number of infected plants, as determined by isolation of *V. dahliae* from the haulms, was a measure of the transmission of this fungus via the tuber.

Influence of year to year variation on percent infection of tubers by *V. dahliae*. In the spring growing seasons from 1978 to 1982, various cultivars were planted in heavily infested plots (~40 microsclerotia per gram of soil) at the Gilat Experiment Station. At the end of the growing season, tubers (60–90 g each) from 50 hills were assayed for internal tuber infection by isolation procedures. In the 1982 season, tubers from two cultivars, Desiree and Spunta, were harvested at the end of the growing season. Seed tuber infection was determined at harvest and from samples kept in storage at 4 C for 10 wk.

Relation between cultivar, foliar symptom expression, or yield and tuber infection by *V. dahliae*. In the spring of 1981, 73 cultivars grown in replicated blocks in soil infested at a similar level as in the previous section were examined for wilt symptoms, total yield, marketable yield (tubers >60 g), percentage marketable yield, and percent tuber infection. Statistical analysis was performed to determine if there was a correlation between any of the first three factors examined and tuber infection.

For isolation of *V. dahliae* from tubers, the tubers first were thoroughly washed and then placed in a solution of 0.5% sodium hypochlorite for 10 min. The stolon end was cut off with a sterile knife to expose the vascular bundles. Three samples of tuber pieces with vascular tissue were plated on a selective medium containing 0.2% sorbose, 100 μg of streptomycin sulfate per milliliter, and 2% agar in 1 L of H_2O. Potato haulms were sampled 10 cm above the soil surface, treated as above, and isolations were made from the vascular tissues. The plates were incubated at 25 C for 12 days, when *V. dahliae* could be readily identified on the basis of microsclerotial formation. Foliar symptom expression was recorded on a scale of 0–4, in which 0 = no symptoms and 4 = dead plant. Classes 1, 2, and 3 represent increasing degrees of chlorosis and necrosis.

TABLE 1. The occurrence of symptom expression of *Verticillium dahliae* on potato, isolation from haulms, percent tuber infection, and the effect of tuber infection on percent infected plants in the subsequent crop

Year	Cultivar	Sample size	Infected plants[a] Symptoms present (%)	Infected plants[a] Stems infested (%)	Daughter tubers[b] (%)	Infection of the subsequent crop[c] (%)
1980	Blanka	880	50	37.0	0.25	0
1981	Desiree	540	2	3.0	11.0	8.0
1981	Desiree	370	80	30.0	1.4	0.5
1981	Alpha	500	15	7.5	5.0	5.0
1981	Spunta	500	12	1.0	7.0	5.0
1982	Spunta	120	0	3.0	11.6	10.0
1982	Pentland Crown	120	0	1.5	21.6	12.0
1982	Desiree	480	100	95.0	36.0	17.1

[a] Infected plants in the spring crop, determined 100 days after planting.
[b] Infection in the spring crop daughter tubers that were used as seed tubers.
[c] From 1,000 hills grown during the autumn season.

TABLE 2. Percentage of infected tubers in 11 potato cultivars during a 5-yr test period at Gilat, Israel

Cultivar	1978	1979	1980	1981	1982
Desiree	12	44	19	36	29
Spunta	16	100	29	14	38
Blanka	24	36	60	20	19
Up-to-Date	35	90	29	16	66
Kondor			7	0	30
Alpha			26	54	35
Patroness			43	72	60
VK 505			9	8	20
Croft			60	60	4
Maris Bard			38	16	28
Cardinal			41	20	6

RESULTS

Relation between foliar symptoms, percent isolation of *V. dahliae* from haulms, and tuber infection. The relation between symptom expression or percent isolation of *V. dahliae* from the haulms and daughter-tuber infection is presented in Table 1.

In one case (cultivar Blanka), although there was a high percentage of haulm infection, there was very little tuber infection which in turn led to 0% infection in the plants grown from these seeds. In contrast, in three cases (Desiree in 1981, Spunta in 1982, and Pentland Crown in 1982) visual examination showed only 0–2% infected plants, but tuber infection ranged from 11–22% which led to relatively high haulm infection in the subsequent autumn-grown plants (8, 10, and 17%, respectively).

Influence of year-to-year variation on percent infection of tubers by *V. dahliae*. The annual variation in percentage of tuber infection during five spring growing seasons is presented in Table 2. Nutritional factors were kept constant, irrigation was supplied as needed, and pathogenic nematodes were not present in the soil. In all five years, inoculum potential was very high (~40 microsclerotia per gram of soil) which according to previous results (*unpublished*) induced 100% haulm infection. However, tuber infection within cultivars varied considerably among years. For example, infection varied from 14 to 100% in cultivar Spunta, 43–72% in Patroness, and 8–20% in VK 505.

The results of storage on percent infection for cultivars Desiree and Spunta indicated that there was no decrease in recovery of the fungus after 10 wk of storage at 4 C. Infection ranged from 24 to 29% in Desiree and from 35 to 38% in Spunta before and after storage.

Relationship between cultivar, foliar symptom expression, or yield and tuber infection by *V. dahliae*. The correlation coefficients between foliar symptoms, total yield, marketable yield, percent marketable yield, and tuber infection of 73 cultivars grown in replicated blocks were −0.42, −0.28, 0.16 and −0.21, respectively. These results show that such commonly used criteria as symptom expression on haulms is not a useful indicator of tuber infection. Furthermore, tolerance, as expressed by increased marketable yield, is also unrelated to the ability of the fungus to penetrate the tuber and establish a detectable infection.

DISCUSSION

Although the role of the potato seed tuber as the disseminating agent of *Verticillium* spp. has been known for some time, very few accounts of the factors leading to tuber infection are found in the literature. Easton (1) found that a large percentage of seed-tuber lots imported into Washington State carried various *Verticillium* spp. either internally or in the soil adhering to the tubers. The latter could be easily killed by a variety of chemicals (1). More recently, Sampson (7) in Australia, showed that tubers of the highly susceptible cultivar Kennebec infected with *V. albo-atrum* gave rise to infected plants, and that the haulms served as a source for inoculum buildup for potatoes sown subsequently.

In our study, we attempted to determine which methods might be useful in determining tuber infection in our spring crop, taking into consideration that the imported seed tubers used for this sowing are free of the pathogen (*unpublished*). Thus, an attempt was made to determine if we could correlate cultivar tolerance, which was exemplified in some of the cultivars that were tested, and tuber

infection.

We also determined survival of the pathogen in tubers during storage, and found, in agreement with Thanassalopoulus and Hooker (9) that survival does not appreciably decrease. Our results demonstrated that neither tolerance nor susceptibility necessarily had a bearing on tuber infection. Also there seem to be large seasonal variations in percent tuber infection in a given cultivar (Table 2) due, perhaps, to temperature and other, as yet unknown, factors. That there can be cultivar differences is to be expected, as shown by the results presented here. For example, in cultivar Mirka, which is grown in our sandy soils, haulm invasion occurs late in the season and tuber infection does not occur (2). With the exception of such an extreme case, tuber invasion seems to be dictated by a number of factors, and from our results it may be stated that the commonly used method of estimating tuber infection by foliage symptomatology is often misleading. This may be especially true when certifying fields for seed where the haulms are killed early to regulate tuber size. Thus, we may ask whether the average yearly total rejection of 0.28% for potato seed certification in the USA and Canada between 1968 and 1972 due to *Verticillium* (8) is truly representative. A possible indication that it may not be realistic is provided by an instance in which, for a seed lot of cultivar Kennebec obtained from one of the above countries and certified as having $<1\%$ wilt, we obtained 25% recovery of *V. albo-atrum*.

Our results also present evidence (Table 1) as to the role of infected tubers in dissemination of *V. dahliae* and lead us to suggest that, in light of the importance of the potato tuber in the disease cycle, certification should be based on the final product sold, ie, the tuber. It should perhaps be mentioned here that in several European countries small seed lots from individual grower's fields are routinely examined for several fungal pathogens. With that method, isolations of *Verticillium* spp. could be made and the pathogens identified. However, we realize that this method would be time consuming and some expertise would be needed to identify the fungi recovered. An alternative rapid method is suggested by our results, which show that serological methods can be used to differentiate among the various *Verticillium* spp. that may be infecting seed potato tubers (5). Using this method we detected specific extracellular metabolites secreted by the fungus in the xylem bundles. Such an approach places these organisms in the category with other tuberborne pathogens, such as bacteria and viruses, that may be detected by serology.

LITERATURE CITED

1. Easton, G. O., Nagle, M. E., and Baily, D. L. 1972. *Verticillium albo-atrum* carried by certified seed potato into Washington and control by chemicals. Am. Potato J. 49:397-402.
2. Krikun, J., and Orion, D. 1979. Verticillium wilt of potato: Importance and control. Phytoparasitica 7:107-116.
3. Krikun, J., Orion, D., Nachmias, A., and Reuveni, R. 1983. The role of soilborne pathogens under conditions of intensive agriculture. Phytoparasitica 10:247-258.
4. MacGarvie, Q. D., and Hide, G. A. 1966. *Verticillium* species from potato seed stocks in Britain in 1965. Plant Pathol. 15:72-75.
5. Nachmias, A., Buchner, V., and Krikun, J. 1982. Differential diagnosis of *Verticillium dahliae* in potato with antisera to partially purified pathogen-produced extracellular antigens. Potato Res. 25:321-328.
6. Robinson, D. B., Larson, R. H., and Walker, J. C. 1957. Verticillium wilt of potato. Univ. Wisconsin Res. Bull. 202. 49 pp.
7. Sampson, P. J. 1980. Infection of Kennebec potato with *Verticillium albo-atrum*. Aust. J. Agric. Res. 31:525-532.
8. Shepard, J. F., and Claflin, L. E. 1975. Critical analyses of the principles of seed potato certification. Annu. Rev. Phytopathol. 13:271-293.
9. Thanassoulopoulus, C. G., and Hooker, W. J. 1968. Factors influencing infection of field-grown potato by *Verticillium albo-atrum*. Am. Potato J. 45:203-216.