

Recovery of *Verticillium dahliae* from Weed Plants in Farmers' Fields in Peru

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ABSTRACT

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Sixty-five weed species from fields used in rotation for potato production were collected from 23 fields in 15 localities in the Mantaro Valley (elevation 3,200–4,200 m) in central Peru. Eighteen of the 65 weed species had *Verticillium dahliae* in their vascular system under natural field conditions. Ten of these did not express any *Verticillium* wilt symptoms; the other eight showed symptoms of chlorosis in the lower leaves, defoliation, and wilt. Presence or absence of external symptoms in weeds artificially inoculated in the greenhouse agreed with those observed under natural field conditions. Volunteer potato plants were infected with *V. dahliae*. There was no relationship between altitude and the weed species infected with *V. dahliae*; the same weed species that are infected with the fungus at the lower agroecological zone (elevation 3,200–3,500 m) also act as a host in the intermediate zone (elevation 3,500–3,800 m). *V. dahliae* was the only *Verticillium* species recovered from weed hosts in the Mantaro Valley.

Verticillium wilt of potatoes (*Solanum tuberosum* L.) was first reported in Peru in 1958, occurring mostly in the coastal valleys (1). Torres and Gutierrez (13) in 1981 and Martin (8) in 1984 reported that the disease was widespread in the highlands and that the causal agent was *Verticillium dahliae* Kleb. Recent studies conducted in the Mantaro Valley (elevation 3,200–4,200 m), where more than 20,000 ha of potatoes are planted annually, indicate that *V. dahliae* is commonly present in potato plants, although heavy yield losses and premature death of plants are not severe because low temperatures and heavy rains during the growing season restrict infection (12). These studies also indicated that in the lower agroecological zone of the valley (3,200–3,500 m) symptoms of *Verticillium* wilt were observed in 97% of the potato fields, and *V. dahliae* was isolated from 55% of the stems collected. On the other hand, in the intermediate zone (3,500–3,800 m), symptoms of *Verticillium* wilt were observed in 25% of the potato fields and the fungus was isolated from only 28% of the stems collected.

Weeds have been mentioned in several reports as one of the means by which *Verticillium* sp. can survive between potato plantings. Furthermore, reports have mentioned that adequate control of weeds would also be required where crop rotation was used to reduce the inoculum potential of the fungus (2,3,11). Although farmers in the Mantaro Valley practice rotations with different crops and control weeds, weeds are normally present in fields that are fallowed for a few months.

In this paper, we report results of a study conducted in the Mantaro Valley in which different weed species were collected. The main objectives were to determine the host range of *V. dahliae* under agricultural conditions and to verify previous reports that *V. dahliae* is the only species causing *Verticillium* wilt in the Mantaro Valley.

MATERIALS AND METHODS

Weed plants were collected from fields where a high incidence of *Verticillium* wilt had been recorded in the potato crop the previous season (12). Weeds were considered plants out of place in the rotation program. Samples were collected from fields located in the low (3,200–3,500 m) and intermediate (3,500–3,800 m) agroecological zones of the Mantaro Valley (6,9,12) during the rainy season, between October 1984 and April 1985. Weed plants were collected from 18 fields in 12 localities in the low zone and from five fields in three localities in the intermediate zone. Ten plants of each weed species were collected per field; five were used for isolation of the pathogen and the other five for plant identification. Identification of weed species was done with the collaboration of the staff of the Botany Departments of the National Agrarian University, Lima, and the National University of Central Peru at Huancayo. The fields from which samples were collected were visited every 30 days to observe newly emerged plants and the development of *Verticillium* wilt symptoms in older plants. Stem sections 10–15 cm long were collected above the soil line, taken to the laboratory, and kept at 4–6 C for 1–2 wk until isolations were performed. Stems were washed in tap water for 15 min, surface-sterilized with 2% sodium hypochlorite solution

for 3 min, and washed twice with sterile distilled water. They were cut longitudinally with a sterilized blade, then the vascular system observed for discoloration and small pieces (0.2–0.4 cm) plated on agar-alcohol medium (10). Plates were incubated at 25 C for 6–8 days, and fungal colonies were studied; those of *Verticillium* sp. were left at the same temperature for an additional week to observe the formation of microsclerotia (4,7,8,11).

Fungal colonies obtained from each plant species were kept separate, and their pathogenicity was tested on potatoes. Thirty 5-day-old potatoes of cultivar Revolución were inoculated with each of the isolates of *V. dahliae* obtained from the weed species by pouring on top of the soil of each pot (3 k of soil) 600 ml of a suspension of 40,000 conidia per milliliter. Plants were kept at 18–28 C for 75 days until symptoms were evaluated and isolation of the fungus was done from the vascular tissue of each plant.

Seeds from most of the weeds were also collected at the end of the season and sown in the greenhouse for artificial inoculation studies. Thirty-day-old seedlings of 11 weed species found infected with *Verticillium* sp. under field conditions were inoculated by immersing their roots for 10 min in a suspension of 15,000 conidia/ml of *V. dahliae*. The fungus isolate used in this test was the most pathogenic on potato according to the results obtained in the test previously mentioned. Inoculated seedlings were then transplanted to individual clay pots containing pasteurized soil and kept in a greenhouse at 15–23 C for 120 days. Three replicates of five seedlings per weed species were inoculated. Roots of five plants of each species were dipped in tap water and served as uninoculated controls. Thirty days after inoculation, small pieces (0.2–0.4 cm) of the vascular tissue of one plant per replicate were plated on agar-alcohol medium to observe the presence of *Verticillium* sp. External symptoms of *Verticillium* infection were recorded weekly until 120 days after inoculation, when all plants were cut at soil level and small portions of their vascular tissue were also plated on agar-alcohol to check for the presence of *Verticillium* sp.

RESULTS

Of the 23 fields sampled only four were replanted with potatoes, two were fallow, and the remainder had a variety of crops,

of which the most common were maize, carrots, alfalfa, peas, oats, and quinoa (*Chenopodium quinoa* Willd.). The total number of plants collected per weed species varied substantially from field to field, making the number of plants for laboratory analysis extremely variable, e.g., *Bidens pilosa* L. was found in 16 fields (80 plants for laboratory assay) compared with *Tagetes elliptica*, which was found in two fields (10 plants for laboratory assay).

Fungal development on the plates 6–8 days after isolation was sufficient to permit easy identification of *Verticillium* sp. All the colonies were later identified as *V. dahliae*; microsclerotia were clearly observed 14 days after isolation in all cases studied (4,7,11).

V. dahliae was recovered from 18 of the 65 weed species collected (Table 1). Ten of these did not have any *Verticillium* wilt symptoms in the field; some of them had only their vascular system discolored. Symptoms in the other eight included chlorosis of the lower leaves, defoliation, and wilt (Table 2). Symptom severity increased with plant age. The highest percentage (50%) of infected plants occurred in *Lamium amplexicaule* L. and the lowest (4%) in *C. quinoa*, *Stachys arvensis* L., *Malva sylvestris* L., and *Veronica persica* L. (Table 1). There was no relationship between altitude and the weed species infected with *V. dahliae*; the same weed species that are infected with the fungus in the lower agroecological zone also act as hosts in the intermediate zone.

Of the 47 weed species collected from which *V. dahliae* was not recovered, only 24 weeds were identified because of lack of competent personnel and their availability in the country that could give a positive identification of the weed. The following weeds were identified: *Amaranthus hybridus* L., *A. clorastachys* L., *Avena sterilis* L., *A. sativa* L., *Brassica oleraceae* L., *Daucus carota* L., *Euphorbia* sp., *Hordeum vulgare* L., *Lupinus mutabilis* L., *Medicago sativa* L., *Melilotus officinalis* Lam., *Oenothera rosca* L., *Pennisetum clandestinum* Hochst., *Phaseolus vulgaris* L., *Pisum sativum* L., *Rumex crispus* L., *Salvia sagittata* L., *Triticum vulgare* L., *Ullucus tuberosus* Lozano, *Urtica urens* L., *Verbena officinalis* L., *Vicia faba* L., and *Zea mays* L.

During the survey, several samples of volunteer potato plants were collected, and all were infected with *V. dahliae*.

All the isolates obtained from weed species were *V. dahliae* and caused *Verticillium* wilt symptoms in potato plants of cultivar Revolución when inoculated artificially. Thirty days after inoculation, chlorosis on the lower leaves was clearly observed, and by the 75th day, most plants were chlorotic, wilted, and defoliated in their lower parts. *V. dahliae* was recovered from the vascular

tissue of all the potato plants. *V. dahliae* also was recovered from the vascular systems of all the 11 weed species that were inoculated and kept in the greenhouse for 120 days. Eight of the species developed external symptoms of the disease that in most cases expressed itself as chlorosis in the lower leaves and defoliation (Table 2). The first species to show symptoms, 30 days after inoculation, was *Brassica campestris*, which had wilt symptoms. Three of the species that developed chlorosis of the lower leaves did not have discolored vascular tissue.

Check plants whose roots were dipped in tap water did not show any external or internal symptoms, and the fungus could not be isolated from the vascular system.

There was complete agreement in the presence or absence of external symptoms between the artificially and naturally infected plants. *Bidens pilosa*, *Tagetes multiflora* L., and *T. elliptica*, which did not show any external symptoms in the field, were also symptomless in the greenhouse. The other eight weed species included in the greenhouse test showed external symptoms in both situations.

Table 1. Weed hosts grown in farmers' fields in the Mantaro Valley, Peru, from which *Verticillium dahliae* was isolated

Plant family and species	Samples collected (no.)	Isolation frequency (%)	External plant symptoms
Compositae			
<i>Bidens pilosa</i> L.	80	11	None
<i>Senecio vulgaris</i> L.	80	20	Chlorosis, wilt, defoliation
<i>Tagetes elliptica</i> L.	10	20	None
<i>T. foeniculacea</i> L.	15	13	None
<i>T. multiflora</i> L.	35	6	None
Cruciferae			
<i>Brassica campestris</i> L.	80	11	Chlorosis, wilt, defoliation
<i>Capsella bursa-pastoris</i> (L.) Medik.	60	28	Chlorosis
<i>Diplotaxis muralis</i> DC.	40	28	Chlorosis, wilt, defoliation
Geraniaceae			
<i>Erodium cicutareum</i> (L.) L'Herit	90	6	None
Labiatae			
<i>Lamium amplexicaule</i> L.	10	50	None
<i>Stachys arvensis</i> L.	45	4	Chlorosis, wilt, defoliation
Leguminosae			
<i>Medicago hispida</i> Gaertn.	95	5	None
Malvaceae			
<i>Malva sylvestris</i> L.	100	4	None
Primulaceae			
<i>Anagallis arvensis</i> L.	10	20	Chlorosis
Portulacaceae			
<i>Calandrineia ciliata</i> Kunth	5	20	None
Chenopodiaceae			
<i>Chenopodium quinoa</i> Willd. ^a	85	4	Chlorosis, wilt
Scrophulariaceae			
<i>Veronica persica</i> L.	65	4	Chlorosis
Solanaceae			
<i>Solanum nigrum</i> L.	5	20	None

^aNot a weed but a crop mentioned as such in the rotation system.

Table 2. External and vascular symptoms of weed species inoculated with *Verticillium dahliae* (root dipping in a suspension containing 15,000 conidia per milliliter) under greenhouse conditions (15–23 C)

Plant species	Symptoms	
	External	Vascular ^a
<i>Anagallis arvensis</i> L.	Chlorosis lower leaves	None
<i>Bidens pilosa</i> L.	None	Brownish discoloration
<i>Brassica campestris</i> L.	Chlorosis, wilt, defoliation	Brownish discoloration
<i>Capsella bursa-pastoris</i> (L.) Medik.	Chlorosis lower leaves	Brownish discoloration
<i>Chenopodium quinoa</i> Willd.	Chlorosis, defoliation	Brownish discoloration
<i>Diplotaxis muralis</i> DC.	Chlorosis lower leaves	None
<i>Senecio vulgaris</i> L.	Chlorosis, defoliation	Brownish discoloration ^b
<i>Stachys arvensis</i> L.	Chlorosis lower leaves	None
<i>Tagetes elliptica</i> L.	None	Brownish discoloration
<i>T. multiflora</i> L.	None	Brownish discoloration
<i>Veronica persica</i> L.	Chlorosis lower leaves	Brownish discoloration

^aData collected 120 days after inoculation.

^bMicrosclerotia present in the stem.

DISCUSSION

There have been several reports on the occurrence of weeds that are hosts of the pathogen causing Verticillium wilt of potatoes and on their importance as one of the means to perpetuate the fungus in the field within a rotation program (2,3,11,14). Most of these investigations, however, were performed by artificially inoculating plants under controlled environments. Reports obtained in this study give additional information on the importance of weed plants as hosts of *V. dahliae* under field conditions and on the need to have effective weed control. The importance of eliminating volunteer potato plants is also indicated in this study by the fact that the pathogen was recovered from volunteer potatoes in several fields. Results obtained by Brown and Wiles (3) and Woolliams (14) on the need for adequate control of weeds to reduce *V. dahliae* soil population and on the importance of symptomless weed plants are confirmed in this research. Ten weed species were found infected with *V. dahliae* although no visible symptoms were observed throughout the growing season (Table 1).

Capsella bursa-pastoris (L.) Medik. and *Solanum nigrum* L. have been reported in previous investigations to

become infected by *V. dahliae* after artificial inoculation (2); the high frequency of isolation of the pathogen from plants of those species grown in farmers' fields (28 and 20%, respectively) confirm their importance as a reservoir of the pathogen (Table 1). Other weed species of genera *Lamium*, *Chenopodium*, *Medicago*, and *Tagetes* have also been reported to host *V. dahliae* (5,14).

Potatoes are a very important crop in the Mantaro Valley (6,8,9), and Verticillium wilt is a common disease regardless of the rotation program (8,12,13), possibly because farmers practice poor weed control in their potato fields. They usually fallow the land for one season and leave it full of weeds, which are later incorporated into the soil during land preparation.

Finally, this study has confirmed previous reports that *V. dahliae* is the only species present in the Mantaro Valley causing Verticillium wilt.

LITERATURE CITED

1. Bazan de Segura, C. 1958. Verticillium wilt of la papa en el Perú. Minist. Agric. Estac. Exp. Agríc. La Molina, Lima, Perú, Bol. 69:1-7.
2. Blush, L. U., Wiles, E. A., and Elango, F. N. 1978. The effect of weeds on the value of rotation as a practical control of Verticillium wilt of potato. Can. Plant Dis. Surv. 58:61-64.
3. Brown, F. H., and Wiles, A. B. 1970. Reaction of certain cultivars and weeds to a pathogenic isolate of *Verticillium albo-atrum* from cotton. Plant Dis. Rep. 54:508-512.
4. Commonwealth Mycological Institute. 1970. Descriptions of pathogenic fungi and bacteria. Nos. 255, 256, 257, 258, and 260. Kew, Surrey, England.
5. Hine, R. B. 1984. Verticillium wilt of *Tagetes* spp. Plant Dis. 68:1098.
6. Horton, D. E. 1984. Social scientists in agriculture research: Lessons from the Mantaro Valley Project, Peru. IDRC, Ottawa, Ontario, Canada. 67 pp.
7. Isaac, I. 1967. Speciation in *Verticillium*. Annu. Rev. Phytopathol. 5:201-222.
8. Martin, C. 1985. Verticillium wilt of potato in central Peru. Am. Potato J. 62:195-199.
9. Mayer, E. 1979. Land use in the Andes: Ecology and agriculture in the Mantaro Valley of Peru with special reference to potatoes. International Potato Center, Lima, Peru. 115 pp.
10. Nadakaruka, M. J., and Horner, C. E. 1959. An alcohol agar medium selective for determining *Verticillium microsclerotia* in soil. Phytopathology 49:527-528.
11. Pegg, G. E. 1974. *Verticillium* diseases. Rev. Plant Pathol. 53:157-182.
12. Pinillos, O., Galindez, W., and Martin, C. 1986. Occurrence and distribution of Verticillium wilt of potatoes caused by *V. dahliae* in the Mantaro Valley, Peru. (Abstr.) Fitopatología 21:9.
13. Torres, H., and Gutierrez, H. 1981. *Verticillium dahliae* Kleb., identificación y síntomas que produce en seis cultivares comerciales de papas peruanas. Fitopatología 16:60-68.
14. Woolliams, G. E. 1966. Host range and symptomatology of *Verticillium dahliae* in economic, weed and native plants in British Columbia. Can. J. Plant Sci. 46:661-669.