Phialophora asteris f. sp. *helianthi*, a New Pathogen of Sunflower in Italy

L. Tosi and **A. Zazzerini,** Institute of Plant Pathology, University of Perugia, Borgo XX Giugno, 74,06100 Perugia, Italy

ABSTRACT

Tosi, L., and Zazzerini, A. 1995 *Phialophora asteris* f. sp. *helianthi*, a new pathogen of sunflower in Italy. Plant Dis. 79:534-537.

A wilt disease was observed during the summer of 1993 in a sunflower field near Perugia in central Italy. The disease was characterized by yellowing of leaves as plants approached flowering. Large areas of the leaves turned light green, usually beginning at the apex and margins and extending inward. Subsequently, leaf margins turned necrotic, and diseased plants were stunted with smaller heads. Our trials proved that this disease was caused by *Phialophora asteris* f. sp. *helianthi*. The fungus was examined for colony morphology, temperature growth responses, and pathogenicity. Significant differences were observed among various culture media. Best growth and profuse sporulation were obtained on carrot and Czapeck agars added with peptone. The optimum temperature for growth was 25 C; temperatures of 5, 10, 15, and 30 C greatly reduced colony growth and sporulation. No growth was observed at 35 C. Significant differences in susceptibility to *P. a. helianthi* were observed in 17 sunflower cultivars tested in greenhouse trials; sunflower lines HA 335, 803-1 (=DM-5), HA 89, HA-RI, and RHA 274 were the most resistant. This is the first report of the disease in Italy and Europe.

Additional keywords: Phialophora yellows, wilt

Sunflower (*Helianthus annuus* L.) is an important crop in Italy; cultivation is concentrated in the central and southern regions. In 1993 120,000 ha of sunflower was cultivated, producing 278,900 t (8).

A disease survey of Umbria sunflower has been conducted since 1980, providing a current picture of the sunflower diseases in this region and their significance. Field inspections during the summer of 1993 found sunflowers (hybrid Montenuovo) with symptoms of a wilt disease in a field near Perugia in central Italy. Yellowing of leaves was noted when plants approached flowering. Large areas of the leaves turned light green, usually beginning at the apex and margins and extending inward. Subsequently, leaf margins turned necrotic, with yellowing around the edges. The lower leaves, between the second and fifth pairs, were dry and withered, whereas the upper leaves remained green. Diseased plants were stunted with smaller heads (Fig. 1). Microscopic observations revealed hyaline hyphae in the vascular tissues of stems.

The present study was undertaken to determine the cause of this disease and evaluate several sunflower lines for disease resistance.

MATERIALS AND METHODS

Isolation. Small tissues taken from sunflower roots and stems were soaked in 1‰ (v/v) sodium hypochlorite for 30 s, washed in sterile distilled water, transferred to potato-dextrose agar (PDA), and incubated at 25 C. Pieces of mycelia from the colonies that developed on PDA were transferred to fresh PDA, and single-spore subcultures from these colonies were recovered and stored in PDA slants at 15 C.

Identification. The isolate was identified on the basis of colony morphology, mycelial characteristics, growth rate, and growth temperatures.

The fungus growth rate was determined on seven culture media—PDA, MA (malt agar), CA (carrot agar), V8 (V8-juice agar), CZA (Czapeck agar), CZA + P (CZA added with peptone, 3g/L), and CZA + YE (CZA added with yeast extract, 2g/L)—all at pH 5.4. Agar inoculum disks (4 mm diameter) were transferred from 2-wk-old PDA cultures in petri dishes of each medium and incubated in darkness at 22 C. Radial growth of the colonies was measured (two measurements at right angles) every 6 days up to 24 days. The trial was repeated twice, with eight replicate dishes each. The data were analyzed using analysis of variance, and Duncan's multiple range test was used to separate means.

The isolated fungus also was tested for mycelial growth on PDA plates incubated in the dark at temperatures from 5 to 35 C, with 5 C increments. All plates were inoculated as described above, and radial growth of the colonies was measured every 3 days up to 15 days. Where no growth occurred, the plates were placed again in an incubator at 20 C to determine whether the temperature was lethal. The experiment was conducted twice, and six replicates per temperature were made. Analysis of variance of the data was performed and significantly different means were separated by Fisher's protected LSD.

Pathogenicity tests. Inoculum was produced on PDA plates incubated at 20 C for 20 days in the dark. Conidia were washed from cultures with 100 ml of sterile deionized water, and the suspension was filtered through a single layer of muslin. The concentration of conidia was determined by hemacytometer and adjusted with sterile deionized water to 10^6 conidia per milliliter.

Table 1. Susceptibility of sunflower lines to *Phialophora asteris* f. sp. *helianthi* in the greenhouse

Lines	Plants in each disease category (%)[x,y]				Percentage of control height[z]
	0	1	2	3	
HA-R1	0	10	90	0	66 f
HA-R2	0	0	100	0	39 a–c
HA-R3	0	30	70	0	36 ab
HA-R5	0	10	80	10	38 a–c
P386	0	50	50	0	46 a–d
RHA 265	0	20	80	0	45 a–d
RHA 274	0	40	60	0	57 d–f
RHA 340	0	40	60	0	40 a–d
RHA 325	0	0	90	10	29 a
HA 335	0	100	0	0	60 ef
DM-2	0	70	30	0	33 ab
803-1 (=DM-5)	0	100	0	0	55 c–f
Impira Inta Sel 11	0	20	80	0	36 ab
Pergamino 71/535	0	40	60	0	30 ab
Sazen Pena	0	50	50	0	30 ab
HA 89	0	80	20	0	41 a–d
LC 74/75 20602	0	10	90	0	32 ab

[x] Plants were scored for disease symptoms 5 wk after inoculation.

[y] 0 = No disease; 1 = plants stunted compared to control, with little necrosis on the first pair of leaves; 2 = plants stunted compared to control, with a lot of necrosis on the first and second pairs of leaves; and 3 = dead plants.

[z] Percents followed by the same letter(s) are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

Accepted for publication 18 January 1995.

Seeds of sunflower hybrid Montenuovo were surface-disinfested with 1% NaOCl for 2 min, rinsed in sterile distilled water, and individually planted in 9-cm-diameter plastic pots containing steamed perlite. Plants were fertilized daily with 20-5-10 (N-P-K) and grown in a greenhouse at an alternate day/night temperature of 22–20 C, 60–80% relative humidity, and 16 h of illumination at 180 $\mu E\ m^{-2}\ s^{-1}$ PAR (photosynthetically active radiation), for 10 days until the cotyledons had expanded fully. The roots of 10 seedlings were washed free of perlite, immersed in inoculum for 5 and 20 min, and replanted in pots (6,12). Roots of control plants were immersed in sterile deionized water. Plants were observed daily for disease development, and 20 days after inoculation all plants were removed for reisolation and were microscopically examined. The test was repeated twice.

Tests for resistance. Five United States sunflower rust differential inbred lines, HA-RI, -R2, -R3, -R5, and P386 (4); four Argentine lines, Impira Inta Sel 11, Pergamino 71/535, Sazen Pena, and LC 74/75 20602 (1); and eight downy mildew differential lines, RHA 265, RHA 274, RHA 340, RHA 325, HA 335, DM-2, 803-1 (=DM-5), and HA 89 (5), were tested for susceptibility. Seeds of the sunflower rust and downy mildew differential cultivars and lines listed in Table 1 were supplied by T. J. Gulya (USDA, Northern Crop Science Lab, Fargo, ND). Ten seedlings of each line were inoculated with the fungal isolate as described above (10^6 conidia per milliliter for 20 min). All lines were tested twice. Plants were rated for disease 5 wk after inoculation.

RESULTS

Isolation. A fungus was consistently isolated from sunflower roots and stems. Initially, the PDA colonies were hyaline without aerial mycelium, and after 3 wk at 22 C, the mycelium changed to dark brown.

Identification and cultural characteristics of the fungus. On PDA, the colonies were composed of close aggregations of hyphae bearing numerous phialides. Gen-

Fig. 1. A sunflower plant showing typical symptoms of disease caused by *Phialophora asteris* f. sp. *helianthi*.

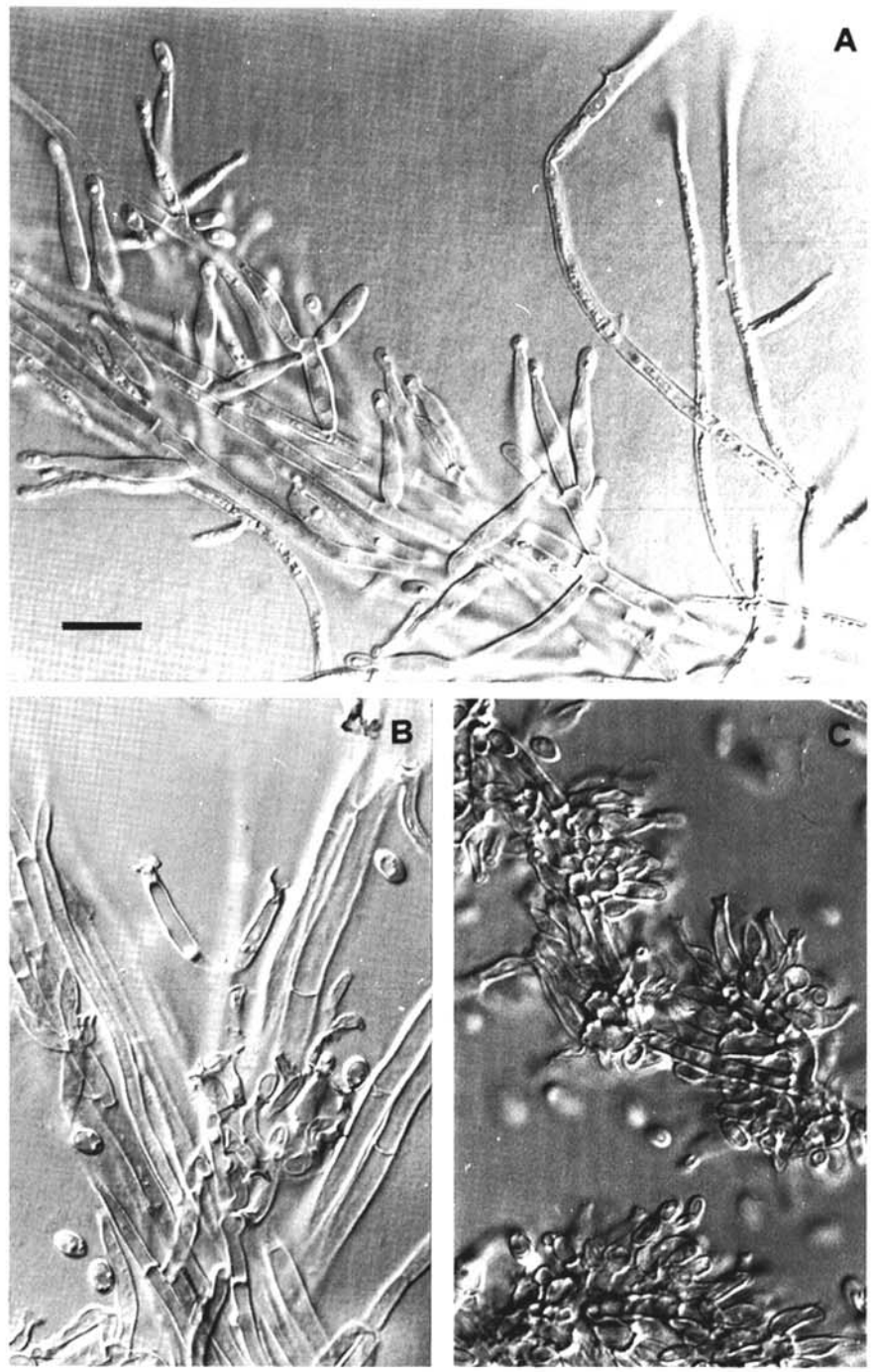

Fig. 2. *Phialophora asteris* f. sp. *helianthi* on various media. **(A)** Phialides and conidia on potato-dextrose agar; **(B)** fascicles of phialides and conidia on V8-juice (V8) agar; **(C)** phialides showing collarettes on V8 agar. Scale bar = 10 μm.

erally phialides were found singly or in groups of two or three on conidiophores; sometimes phialides were found on alternate sides of hypha or in opposite pairs (Fig. 2A). Phialides ranging between 11 and 16 µm (mean 12.6 µm) were slightly swollen in the middle with a small apical collarette (Fig. 2B).

The shape and size of the phialides appeared to be influenced by the medium: on V8 agar they were smaller (7–11 µm), strongly curved, and organized in dense fascicles or bushes (Fig. 2C). Verticillate or penicillate patterns of development were never observed. Conidia that formed singly at the apices of phialides were hyaline and subcylindrical to ellipsoid, usually with two guttula measuring 2.5–7.5 µm (mean 5.7 µm) long and 1.5–2.5 µm (mean 2.0. µm) wide. No chlamydospores or sclerotia were observed. Based on these morphological features, the fungus was identified as *Phialophora asteris* (Dowson) Burge & Isaac f. sp. *helianthi* Tirilly & Moreau. The identity of this fungus has been confirmed by Centraalbureau Voor Schimmelcultures, Baarn, the Netherlands.

Significant differences in growth of mycelia were observed among the various media. Greatest growth of *P. a. helianthi* on culture media was on CA and CZA + P, with average colony diameters of 79.7 and 79.6 mm, respectively, after 24 days of incubation at 22 C (Fig. 3). On PDA and MA, colonies were dark brown with an outer white-cream periphery; on CA and V8, mycelia changed to light brown; and on CZA + P, colonies were white. On these media (PDA, CA, V8, MA, and CZA + P), colonies showed a regular margin and had a bright appearance resembling that of bacterial cultures. Colonies on CZA and CZA + YE showed the least growth (5.7 and 4.3 mm respectively) (Fig. 3), an irregular margin, and on CZA + YE a slightly floccose mycelium.

Sporulation was strongly affected by media. Colonies grown on PDA, CA, CZA + P, and V8 produced abundant phialides and conidia, whereas on the other media (MA, CZA, and CZA + YE), sporulation was poor.

Greatest growth and profuse sporulation occurred within 15 days on PDA at 25 C (52.2 mm). Within a 20–25 C temperature range, colonies of *P. a. helianthi* had dark-brown mycelia and abundant sporulation. Reduced colony growth with white mycelia and less sporulation was observed at 5, 10, 15, and 30 C. No growth occurred at 35 C, but the effect of temperature was not lethal because colonies reincubated at 20 C grew and sporulated normally (Fig. 4).

Pathogenicity tests. Stunting and yellowing of cotyledons were apparent as early as 10 days after inoculation, and the symptoms were more evident and severe on plants inoculated with *P. a. helianthi* for 20 min compared to those inoculated for 5 min. Thirty days after inoculation, twisting of leaves, leaf necrosis, and collapse of cotyledons occurred on all inoculated plants. The root system of inoculated plants was much smaller than that of controls based on visual observation. Stunted plants showed browning of the vascular tissues extending from the roots to the stem. Cross sections of the infected stems showed that the discoloration affected cortical and vascular tissues and that hyphae invaded the vessels. The fungus was subsequently reisolated from roots and stems of inoculated plants, but no organisms were recovered from control plants.

Tests for resistance. All sunflower differential lines tested showed some susceptibility to *P. a. helianthi* with HA 335, 803-1 (=DM-5), HA 89, HA-RI, and RHA 274 being the most resistant, whereas HA-R5 and RHA 325, were the most susceptible (Table 1). *P. a. helianthi* generally caused a reduction in the height of the infected plants compared to controls, but stunting always was associated with leaf necrosis. Therefore, to evaluate cultivar resistance to Phialophora yellows accurately, the data reported in Table 1 must be interpreted according to stunting and the percentage in each category of disease. So HA 335, 803-1 (=DM-5), and HA 89, although not showing the least amount of reduction in the height of infected plants, can be considered the most resistant genotypes, with 100% of infected plants in the first disease category. HA-RI and RHA 274, which show the most stunting and which have a high percentage of infected plants in the second disease category, also can be considered resistant.

DISCUSSION

Yellowing of sunflower caused by *P. a. helianthi* was confined to a small area of a field, and observations carried out during the summer indicated that the Montenuovo plants were moderately or severely infected. Sunflower were grown for 2 yr in this field, and no disease was noted, indicating that weather conditions may play a role in disease development.

Presumably, *P. a. helianthi* is a soilborne pathogen and overwinters in infected plant debris. Little is known about its life cycle, but as with other wilt diseases (e.g., *Verticillium dahliae*), this parasite might be transmitted by seed (10,11). Remnants of Montenuovo seeds used to plant the crop were not available, so isolation of the pathogen from seeds was not possible.

The symptomatology caused by *P. a. helianthi* might be confused with mineral disorders, excess water, or Verticillium wilt. However, symptoms of Verticillium wilt are characterized by yellow interveinal patches, usually in the center or near the periphery of the leaves. The yellow areas enlarge and become brown and necrotic. Ultimately, the entire leaf may turn brown, and the necrotic areas may be bordered by chlorosis (12,14). Since the symptoms of Phialophora and Verticillium wilts are similar and breeders may not initially recognize the differences, it would be appropriate to discuss sources of *Verticillium* resistance. We tested HA 89 (universal suscept to downy mildew races), which is resistant to the North American

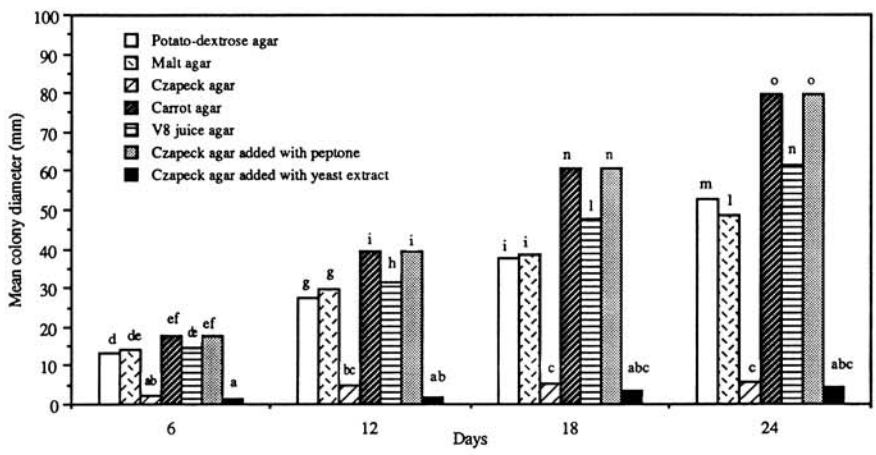

Fig. 3. Radial growth (in millimeters) of *Phialophora asteris* f. sp. *helianthi* on different media at 22 C. Columns with the same letters are not significantly different at $P = 0.05$ according to Duncan's multiple range test. Means are based on eight replications.

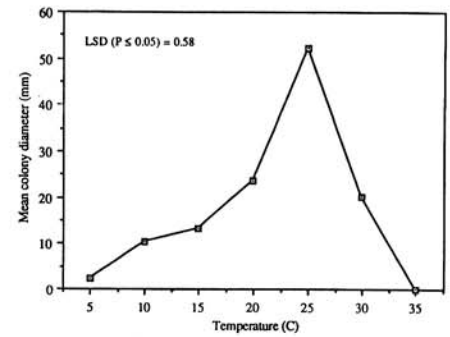

Fig. 4. Effect of temperature on the radial growth of *Phialophora asteris* f. sp. *helianthi* on potato-dextrose agar 15 days after inoculation.

biotype of *Verticillium* (3) but not to the South American biotype (2), for which the hybrid Contiflor 1 is resistant. The lines HA-R4 and -R5, developed for rust resistance, also are resistant to the North American biotype of *Verticillium* (4). Thus, it appears that resistance to *Verticillium* and *Phialophora* is not linked, since HA 89 is tolerant to *Phialophora* and HA-R5 is very susceptible.

Finally, Hoes and Enns (7) investigating the inheritance of *Phialophora* resistance found that the inbred line CM361 is resistant and that resistance to Phialophora yellows is based on two genes, one of which is dominant. CM361 is a selection of the Russian open-pollinated VNIIMK 8931. Korell et al (9) have published a pedigree map in which they show two additional Canadian lines, CM359 and CM303, as selections of VNIIMK 8931. The USDA line HA 89, which had resistance to *Phialophora* in this study, is also a selection of VNIIMK 8931, and HA 335, another resistant entry, is a cross of HA 89 with wild *H. annuus*.

Our isolate of *P. a. helianthi* showed some differences in its morphological and cultural characteristics compared to the Canadian isolate tested by Tirilly and Moreau (13). The Italian isolate grows at temperatures ranging from 5 to 30 C with an optimum of 25 C, whereas the Canadian isolate grows at an optimum 22 C. On synthetic media, our isolate grows most rapidly and sporulates profusely on PDA, MA, CA, V8, and CZA + P, whereas the Canadian isolate grows best on CZA + P and MA.

Yellowing of sunflower was first described in Manitoba, Canada, in 1968 and was caused by *Phialophora* sp. The present report of *P. a. helianthi* on sunflower, the only one since Hoes' work (6), is the first report of Phialophora yellows in Italy and Europe.

ACKNOWLEDGMENTS

This research was supported by the Italian Ministry of University and Scientific and Technological Research (60% grant). We thank M. Orfei for technical assistance. We thank. T. J. Gulya (USDA-ARS, Oilseeds Research Unit, Northern Crop Science Laboratory, Fargo, ND 58105) for providing sunflower differential seeds.

LITERATURE CITED

1. Anonymous. 1988. Report of the Ad Hoc Committee on Sunflower Rust of the International Sunflower Rust Committee meeting. Proc. Int. Sunflower Assoc. Novi Sad, Yugosl. 2:250-255.
2. Bertero, A. B., and Vazquez, A. N. 1982. Nueva raze de *Verticillium dahliae* Kleb. Pages 177-178 in: Proceedings of the 10th International. Sunflower Conference.
3. Gick, G. N., and Zimmer, D. E. 1974. Monogenic resistance to Verticillium wilt in sunflowers. Crop Sci. 14:895-896.
4. Gulya, T. J. 1985. Registration of five disease-resistant sunflower germplasms. Crop Sci. 25: 719-720.
5. Gulya, T. J., Sackston, W. E., Viranyi, F., Masirevic, S., and Rashid, K. Y. 1991. New races of the sunflower downy mildew pathogen (*Plasmopara halstedii*) in Europe and North and South America. J. Phytopathol. 132:303-311.
6. Hoes, J. A. 1972. Sunflower yellows, a new disease caused by *Phialophora* sp. Phytopathology 62:1088-1092.
7. Hoes, J. A., and Enns, H. 1974. Inheritance of resistance to Phialophora yellows of sunflower. Pages 309-310 in: Proceedings of the 6th International. Sunflower Conference.
8. ISMEA. 1994. Statistiche. Olio Grassi Veget. 114:26.
9. Korell, M., Mosges, G., and Friedt, W. 1992. Construction of a sunflower pedigree map. Helia 15:7-16.
10. Sackston, W. E. 1980. Some factors influencing infection of sunflower seed by *Verticillium dahliae*. Can. J. Plant Pathol. 2:209-212.
11. Sackston, W. E., and Martens, J. W. 1959. Dissemination of *Verticillium albo-atrum* on seed of sunflower (*Helianthus annuus*) Can. J. Bot. 37:759-768.
12. Sackston, W. E., McDonald, W. C., and Martens, J. 1957. Leaf mottle or Verticillium wilt of sunflower. Plant Dis. Rep. 41:337-343.
13. Tirilly, Y., and Moreau, C. 1976. Etude comparative de quelques *Phialophora* parasites vasculaires de plantes. Boll. Sc. Mycopathol. Fr. 92:349-358.
14. Zimmer, D. E., and Hoes, J. A. 1978. Diseases. Pages 244-248 in: Sunflower Science and Technology. J. E. Carter, ed. American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Madison, WI.