

## FIRST REPORT OF *Phialophora asteris* f. sp. *helianthi* ON SUNFLOWER IN PAKISTAN

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### SUMMARY

During summer 1997, a wilt disease of sunflower (*Helianthus annuus* L.) was observed in the experimental field at NARC, Islamabad. The diseased plants exhibited symptoms of wilt with dull yellowing of leaves at flowering. Large areas on the leaves first turned dull light green, usually starting at the apex and leaf margins and extending inward, and then became necrotic. Severely infected plants were stunted, with small heads. The causal agent was isolated and, based on the characteristics morphological features, identified as *Phialophora asteris* f. sp. *helianthi*. This is the first report of the disease on sunflowers in Pakistan.

**Key words:** wilt, *Phialophora* f. sp. *helianthi*, pathogenicity, *Helianthus annuus*, Pakistan

### INTRODUCTION

Sunflower yellows, a systemic disease caused by soil-borne organism *Phialophora* sp. was first described in Manitoba, Canada in 1968 (Hoes, 1972). It was recently identified as *Phialophora asteris* (Dowson) Burge & Issac f. sp. *helianthi* (Tirilly and Moreau, 1976). The disease has also been reported from Perugia in central Italy, France and Europe (Hoes and Enns, 1974; Tirilly and Moreau, 1976; Moreau, 1977; Tosi and Zizzerini, 1995). Hoes and Enns (1974), investigating the inheritance of *Phialophora* resistance, found that the inbred line CM-361 was resistant and that the resistance to *Phialophora* yellows was based on two genes, one of which was dominant. A source of resistance is known and transfer of resistance to new cultivars is not difficult.

During summer 1997, a wilt disease was observed in the sunflower (*Helianthus annuus* L.) experiment field at the National Agricultural Research Centre (NARC), Islamabad. The diseased plants exhibited symptoms of wilt, with leaves turning a dull light green color near flowering time. Large areas on the leaves soon turned a dull yellow color, usually starting at the apex and leaf margins and extending inward. Entire leaves assumed a dull yellow color and 5-10 mm long angular

patches of interveinal tissue became necrotic. Leaf margins also became necrotic and necrotic tissue was not surrounded by yellow edges. Severely infected plants were stunted, with small flower heads and the vascular tissue turned brown. These symptoms were similar to those described earlier (Zimmer and Hoes, 1978; Hoes and Enns, 1974; Tirilly and Moreau, 1976; Moreau, 1977; Tosi and Zizzerini, 1995).

The purpose of this study were i) to identify the causal agent, ii) to report its first occurrence on sunflowers in Pakistan and iii) to determine its pathogenicity.

## MATERIAL AND METHODS

### Isolation and identification

Small pieces of tissue from infected roots and stems of sunflower showing wilt symptoms collected from the field at NARC, Islamabad, were surface-disinfected in 1% sodium hypochlorite (NaOCl) for 1 min, blotted dry and then transferred to 9 cm petri plates containing potato dextrose agar (PDA) medium amended with 250  $\mu$ g of streptomycin sulphate/ml (Stevens, 1974) and incubated at 25°C. The hyphal tips from growing colonies developed on PDA were subcultured on fresh PDA to obtain pure culture of the pathogen.

### Pathogenicity

For preparation of inoculum, conidia were harvested from isolates on PDA plates, incubated at 20°C for 20 days in the dark, with 100 ml of sterile distilled water (SDW). The seed of susceptible sunflower hybrid, after surface-disinfection, were individually grown in 9 cm plastic pots in a greenhouse and kept at temperatures of 20-26°C with 60-80% relative humidity for 10-15 days as described by Tosi and Zizzerini (1995). The roots of 10 seedlings were washed and inoculated by dipping in inoculum for 20 min and replanted in pots (Sackston *et al.*, 1957; Hoes, 1972; Tosi and Zizzerini, 1995). Roots of control plants were dipped in SDW. Plants were examined daily for disease symptoms and 20 days after inoculations all the plants were removed for reisolation.

## RESULTS AND DISCUSSION

### Isolation and identification

The casual agent was consistently isolated from the infected sunflower roots and stems. The mycelial colonies that developed were hyaline at initial stage and then, after 3 weeks at 22°C, they changed the color to dark brown. They were composed of close aggregations of hyphae, bearing numerous phialides, which developed on conidiophores singly or in groups of 2-3, measuring 10-15  $\mu$ m (mean 12  $\mu$ m) and were slightly swollen in the middle, with a small collarette. The conidia

that formed singly at the apices of phialides, were hyaline and subcylindrical to ellipsoid, usually with 2 guttula, measuring 2.5-7.5 x 1.5-2.5  $\mu\text{m}$  (Tirilly and Moreau, 1976; Tosi and Zizzerini, 1995). No chlamydospores or sclerotia could be observed. Thus, based on these characteristic morphological features, the causal agent was identified as *Phialophora asteris* (Dowson) Burge & Issac f. sp. *helianthi* Tirilly & Moreau.

### Pathogenicity

Symptoms of severe stunting and yellowing of cotyledons appeared as early as 10 days after inoculation. Conspicuous twisting of leaves, leaf necrosis and collapse of cotyledons were observed in all the inoculated plants after 30 days of inoculation. Root system of the inoculated plants was much smaller than that of the control plants. Stunted plants showed the browning of vascular tissues extending from roots to stem (Tirilly and Moreau, 1976; Tosi and Zizzerini, 1995). The causal agent was consistently reisolated from the roots and stems of inoculated plants while no organism was recovered from control plants.

The symptoms caused by *P. a. helianthi* might be confused with nitrogen deficiency and also resemble those of a mild infection of *Verticillium* wilt but there are characteristic differences. The symptoms of *Verticillium* wilt are characterized by prominent yellow interveinal patches, usually occurring first in the center or near the periphery of leaves. These chlorotic patches enlarge and coalesce while their centers turn brown and necrotic. Ultimately, the entire leaf may turn brown and wither but "halos" of yellow tissue persist around the necrotic areas (Sackston et al., 1957; Zimmer and Hoes, 1978). In *Phialophora* yellows, however, necrotic tissue is not surrounded by chlorosis (Hoes and Enns, 1974; Tirilly and Moreau, 1976; Moreau, 1977).

Although the disease was already reported from Canada, Italy, France and Europe (Hoes, 1972; Hoes and Enns, 1974; Tirilly and Moreau, 1976; Moreau, 1977; Tosi and Zizzerini, 1995), this is the first report of *Phialophora* yellows on sunflowers in Pakistan.

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**PRIMER INFORME SOBRE LA APARIENCIA DE  
PATOGENOS *Phialophora asteris* f. sp. *helianthi* EN EL  
GIRASOL EN PAQUISTAN**

RESUMEN

Durante el verano de 1997, la enfermedad de girasol (*Helianthus annuus* L.) ha sido notada en el campo experimental de NARC, Islamabad. Las plantas enfermas tenían los síntomas de marchitez, con amarillez oscura de hojas en la fase de florecimiento. Las grandes superficies de hojas se hacían primeramente verdes claros oscurecentes, generalmente a partir de la cima y los bordes de hoja al medio, pues aparecía la necrosis. Las plantas infectadas intensamente eran atrofiadas, con cabezas pequeñas. El agente patógeno fue aislado e identificado a base de las propiedades morfológicas características como *Phialophora asteris* f. sp. *helianthi*. Eso es el primer informe sobre la aparición de ésta enfermedad de girasol en Paquistán.

**PREMIER RAPPORT SUR L'APPARITION DU *Phialophora*  
*asteris* f. sp. *helianthi* DANS LE TOURNESOL AU  
PAKISTAN**

RÉSUMÉ

Au cours de l'été 1997, une maladie du tournesol (*Helianthus annuus* L.) a été remarquée dans le champ expérimental de NARC à Islamabad. Les plantes atteintes présentaient des symptômes de flétrissement avec un jaunissement terne des feuilles au moment de la floraison. Une couleur jaune pâle terne s'étendaient sur de grandes parties de la feuille en partant du sommet et en s'élargissant vers le centre avant l'apparition de la nécrose. Les plantes gravement atteintes étaient étiolées et avaient de petites têtes. La cause de la maladie a été isolée et identifiée par ses propriétés morphologiques comme étant le *Phialophora asteris* f. sp. *helianthi*. Ceci est le premier rapport de l'apparition de cette maladie du tournesol au Pakistan.