Two New "Verticillium" Threats to Sunflower in North America

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ABSTRACT

A new strain of *Verticillium dahliae*, the fungus causing Verticillium leaf mottle and wilt, was identified from northwestern Minnesota in 2002. The new strain or biotype is characterized by its ability to overcome the single, dominant V-1 resistance gene employed in oilseed and confection hybrids. Samples collected in 2003 from diseased plants confirmed the new biotype also exists in Manitoba, Canada. All *V. dahliae* isolates collected to date from North Dakota and South Dakota have been identified as the "original" biotype, still fully controlled by the V-1 gene, suggesting that the distribution of the second biotype may be limited to northwestern MN. Another fungus, *Phialophora asteris* f. sp. *helianthi*, was isolated from oilseed sunflower plants showing leaf mottle symptoms in Minot, North Dakota, and presumed to be due to *Verticillium dahliae.* In greenhouse inoculations, *Phialophora* produced leaf mottle symptoms, on sunflower lines with and without the V-1 gene, which were very similar to those incited by *V. dahliae*. Proper identification of this pathogen from plants displaying leaf mottle symptoms will be necessary to avoid confusion between the two pathogens.

INTRODUCTION

Verticillium dahliae causes a disease of mature sunflowers referred to as Verticillium wilt, although the symptoms would more accurately be described as a leaf mottle (Sackston et al., 1957). Genetic resistance, based on a single, dominant gene from wild *Helianthus annuus,* was identified by Canadian researchers (Putt, 1964) and has been incorporated into released inbreds (Fick and Zimmer, 1974), which have been used to produce resistant oilseed hybrids. More recently, confection inbred lines have been released with *Verticillium* resistance conferred by the V-1 gene (Miller and Gulya, 1985). It is unknown what percentage of commercial hybrids has *Verticillium* resistance. A biotype or biotype of *V. dahliae* not controlled by the V-1 gene was identified in Argentina in 1985 (Bertero and Vazquez, 1982). To date, however, only one biotype of *V. dahliae* has been identified on sunflowers in North America.

The incidence of *Verticillium* wilt of sunflower has increased in recent years (Gulya, 2003). In 2002, *Verticillium* was recorded by NSA surveyors in 19% of fields and affected 5.4% of the entire U.S. crop; in 2003, the disease was recorded in 13% of surveyed fields, affecting 2% of the crop. Wilt was only recorded in ND and SD, although the fungus exists across the U.S. In 2002, the incidence of wilt in SD reached 18%, spurring interest in determining the cause of this dramatic increase.

A leaf mottle resembling that caused by *V. dahliae* was observed in Manitoba in the early 1970's, and the causal agent identified as a species of *Phialophora* by Hoes (1974). The disease was also observed in Italy, where researchers identified the causal organism as *P. asteris* ssp. *helianthi* (Tosi and Zazzerini, 1995). No further observations of this disease have been recorded since then in other countries.

MATERIALS AND METHODS

Stalks from plants showing symptoms of *Verticillium* wilt were collected during the annual NSA-sponsored survey in 2002 and 2003, as well as by USDA personnel from other sites. Fungi were isolated in pure culture from the stalks, identified, and pathogenicity proven with greenhouse inoculations, using HA-372 as a *Verticillium* susceptible check and HA-89 (having the V-1 gene) as a resistant line. Greenhouse inoculations employed procedures suggested by sunflower researchers in Argentina, including stem inoculations and root dip inoculations, on 3 to 4 wk old plants. Symptoms developed two to three weeks after inoculation.

RESULTS

One *Verticillium dahliae* isolate, collected from Mentor, MN in 2002, produced typical leaf symptoms in HA-89 in greenhouse tests (Figure 1) , confirming the existence of a new biotype of the fungus. This new isolate was also recovered from stalks collected at Morden, Manitoba. Plants displaying leaf mottle symptoms from Minot, ND yielded a fungus identified as *Phialophora asteris* f. sp. *helianthi*. Stalks from the affected plants did not have dark microsclerotia characteristic of *V. dahliae* infection. *Phialophora asteris* f. sp. *helianthi* (PAH). produced leaf symptoms on greenhouse-inoculated plants nearly identical with that incited by *V. dahliae*, and PAH was able to infect HA-89 (Fig. 2), demonstrating that the V-1 gene does not confer resistance to PAH.

Figure 1. Typical leaf mottle symptoms produced by *Verticillium dahliae* on field grown sunflower.

Figure 2. Leaf mottle symptoms on produced by *Phialophora asteris* f. sp. *helianthi* on sunflower line HA 89 in greenhouse tests.

Differentiation between *V. dahliae* and PHA in culture was easy, based on the presence of small, irregularly shaped, black microsclerotia (50 to 100) of *V. dahliae*, and the absence of microsclerotia with PAH (Figure 3). Additionally, the conidia of *Verticillium* are produced on verticillate whorls while those of *Phialophora* are borne on phialides with collarettes, and the latters conidia are also larger (vs). On symptomatic plants, the easiest way to distinguish *Phialophora* from *Verticillium* is the presence of black microsclerotia on the pith exterior with *Verticillium* infection (Figure 4).

Figure 3. *Verticillium dahliae* (left) and *Phialophora asteris* f. sp. *helianthi* . (right). Dark morphology is due to the presence of microsclerotia

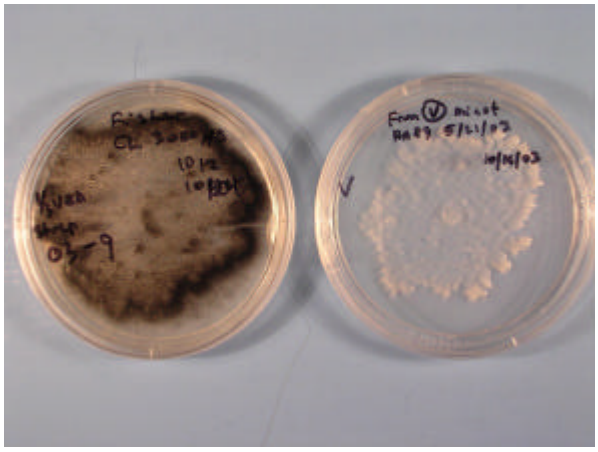


Figure 4. Pith of sunflower plant infected by *Verticillium dahliae* showing typical black discoloration due to microsclerotia.

DISCUSSION

At least two biotypes of *V. dahliae* (Vd) now exist in North America (U.S. and Canada) that infect sunflower. The V-1 gene, a single, dominant gene, does not protect sunflower against either the new isolate of Vd or against PAH. Two species of *Verticillium* occur in North Dakota and Minnesota soils: *V. dahliae* and *V. albo-atrum.* While potatoes are infected by both *Verticillium* species (McKeen & Thorpe, 1981), no one has determined whether *V. albo-atrum* is able to infect sunflower. Foliar symptoms caused by the different biotypes of Vd are indistinguishable, and very similar , but the presence of blackened pith should help identify *V. dahliae* (Fig. 4). Another fungus, *Phialophora asteris* f. sp. *helianthi*, produces symptoms that might be mistaken for a mild *Verticillium* infection, and this fungus has been observed both in North America (Hoes, 1972) and Europe (Tosi & Zazzerini, 1995). Microscopically, *Phialaphora* can be distinguished from Vd by the absence of microsclerotia in Phialophora. The major morphological difference between *Phialophora* and *V. albo-atrum* is the collarettes and the non-verticillate arrangement of the conidia-bearing phialides of *Phialophoa.*

The incidence of Verticillium leaf mottle has been monitored over the last three years by the annual NSA fall survey. Leaf mottle distribution in ND from 2001 to 2003 is depicted in Figures 6, 7 and 8, and illustrates that the disease is likely to occur in any area of the state. Maps showing disease distribution in other states can be accessed at the website: http://134.129.78.3/sunflower/. The incidence of each *Verticillium* biotype, however, has not been determined, and a concerted effort is needed to document this. Coordination between public and private sunflower researchers, coupled with the annual NSA survey, will be needed to accomplish this task.

Fig. 5. Distribution of Verticillium leaf mottle in 2001 in North Dakota, depicted by pink shading. Dots denote fields inspected during fall NSA survey.

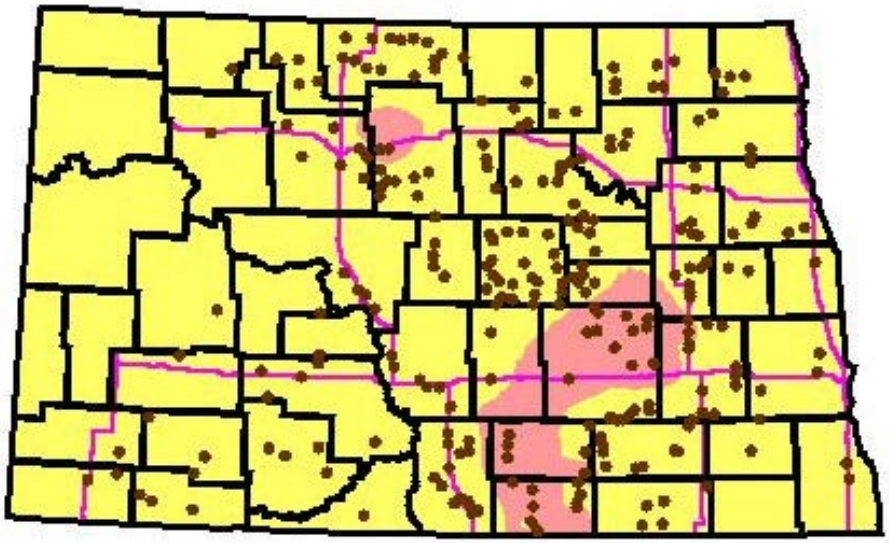


Fig. 6. Distribution of Verticillium leaf mottle in 2002 in North Dakota.

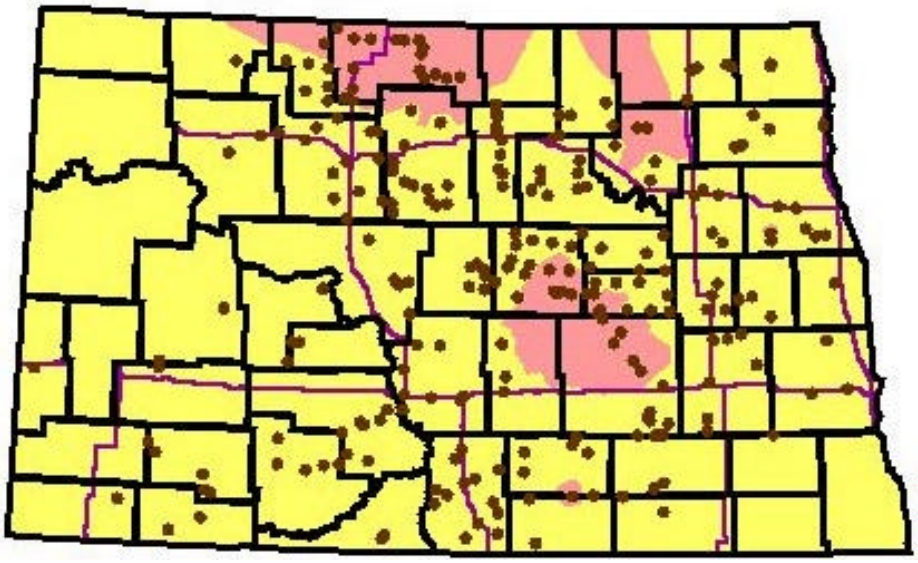
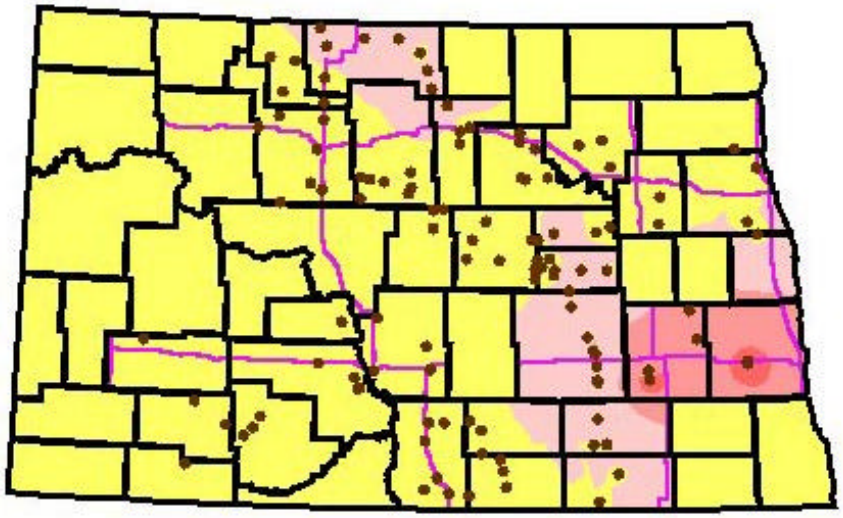


Fig. 7. Distribution of Verticillium leaf mottle in 2003



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