

NEW STRAIN OF *Verticillium dahliae* IN NORTH AMERICA

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SUMMARY

A new strain of *Verticillium dahliae*, the fungus causing Verticillium leaf mottle and wilt, was identified from northwestern Minesota 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. Limited survey, conducted in September 2004, identified the new strain in roughly one-quarter of fields showing Verticillium wilt in North Dakota. In greenhouse trials in 2004/5 we tested 221 cultivars for reaction to the new Verticillium strain. One entry from the USDA Plant Introduction collection, developed in Russia as VNIIMK 8883, was immune and would be an ideal source of resistance. Other commercial oilseed hybrids from the U.S., Argentina and Europe, were also noted with ratings of 0.5 or lower on a 0 to 5 scale. On a related note, another fungus, *Phialophora asteris* F. sp. *helianthi*, was isolated from oilseed sunflower plants showing leaf mottle symptoms in Minot, North Dakota in 2002, 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.

Key words: new strain, *Verticillium dahliae*, northwestern Minesota, Manitoba, North Dakota, *Phialophora*

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 reseachers (Putt, 1964) and has been incorporated into released inbreds (Fick and Zimmer, 1974), which

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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. 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.

MATERIAL 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 week old plants. Symptoms developed two to three weeks after inoculation.

In 2005, a preliminary greenhouse study was initiated to search for resistance to the new North American *Verticillium* strain. A total of 221 entries were tested, most of which were hybrids. The entries were from the FAO 2004 trial, the USDA oilseed and confection trial, and the "core collection" of the USDA Plant Introduction sunflower collection. Plants were inoculated by injecting the stem with a suspension of microconidia at the V-3 stage, with three replications of six plants per replication. Symptoms were monitored for four weeks after inoculation and rated on a 0 to 5 scale. USDA lines HA-89 (known for possessing V-1 gene) and HA-372 (no known *Verticillium* genes) were used as checks.

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 characteristics 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, demonstrating that the V-1 gene does not confer resistance to PAH.

Table 1: Reaction of the most resistant and most susceptible entries in an inoculated greenhouse trial of 221 sunflower cultivars, using the new North American strain of *Verticillium dahliae*. Verticillium rating is on a 0-5 scale, with 5 the most susceptible

Company/Country	Cultivar name	Type	Rating
Most resistant			
PI 307942 – USDA	VNIIMK 8883	Oil	0
ProSeed – USA	9405	Oil	0.2
CHS – USA	Exp 01	Oil	0.3
Seeds 2000 – USA	X 978	Oil	0.3
Ames 18900 – USDA	Stepnyak	Oil	0.5
Nidera – Argentina	GN 4221	Oil	0.5
Maisadour – France	Parma	Oil	0.9
Advanta – Spain	C 70165	Oil	1.3
Red River – USA	2214	Conf	1.8
Hagen – USA	7253	Conf	1.9
Most susceptible			
Dahlgren – USA	D 4421	Oil	1.9
Dekalb – USA	MH 4231	Oil	2
Interstate – USA	Hysun 521	Oil	2.1
Fundulea – Romania	HS 2524	Oil	3.1
Nidera – Argentina	Paraiso 33	Oil	3.1
Advanta – USA	NS 4540	Oil	3.2
Hagen – USA	8232	Conf	3.3
Dahlgren – USA	9530 M	Conf	3.6
Ames 3391	Romsun V3355	Oil	3.8
PI 296289 - USDA	Jupiter	Oil	4

Greenhouse evaluations of 221 sunflower cultivars with the new North American Verticillium strain revealed a wide range of disease reaction. USDA check varieties HA-89 had a rating of 1.9 and susceptible check, HA-372 had a rating of 2.6. HA-89, inoculated with the original North American Verticillium strain was

immune. One entry (P.I. 307942 from the USDA Plant Introduction collection, and originally from Russia as VNIIMK 8883) was symptomless on all plants. Several other currently marketed oilseed hybrids, from the USA, Argentina and Europe, were quite resistant with rating under 1. Confection hybrids exhibited the lowest of resistance to the new strain of *Verticillium*. The most susceptible entry in the trial was an older open-pollinated variety from South Africa (Jupiter) from the USDA Plant Introduction collection.

DISCUSSION AND CONCLUSION

The occurrence of a new strain of *Verticillium dahliae* in North America marks the end of several decades during which the V-1 gene effectively controlled Verticillium wilt. Since the V-1 gene controls neither the new *Verticillium* strain nor *Phialophora*, researchers will need to find resistance genes for both diseases and incorporate them into parental lines for both oilseed and confection hybrids. In our preliminary greenhouse tests, we have identified one immune source (VNIIMK 8883) for resistance to both strains of *Verticillium*. This and other highly resistant entries are currently in field trials, using both naturally-infested fields and artificial inoculations.

Further surveys are planned for 2005 to delineate the geographic distribution and frequency of both *Verticillium* strains. Additionally, once permits are obtained to allow importation of *Verticillium* isolates from Argentina and other countries, we plan to do research on all *Verticillium* strains, including selecting a set of publicly available differential lines, and finding resistance to the new North American strain, and ultimately transferring that resistance into elite inbred lines with high oleic and other necessary agronomic traits. Although the severity and economic impact of Verticillium on US sunflower production is not as great as that observed in Argentina, for example, the USDA will continue in its efforts to delineate the scope of the problem, identify sources of resistance, and develop and release oilseed and confection lines with resistance.

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NUEVO LINAJE DE *Verticillium dahliae* EN AMÉRICA DEL NORTE

RESUMEN

Un nuevo linaje de *Verticillium dahliae*, el hongo que causa marchitez por Verticillium y secado anticipado del follaje, fue identificado en Minesota noroeste, en el año 2002. El nuevo linaje o biotipo caracteriza la capacidad de superación de un gen de resistencia dominante, V-1, que está incorporado en los híbridos oleaginosos y de consumo. Las muestras de las plantas infectadas, recolectadas en el año 2003, confirmaron que el nuevo biotipo existe también en Manitoba, Canadá. Una investigación limitada, llevada a cabo en septiembre de 2004, detectó la presencia de un nuevo linaje en alrededor de una cuarta parte de los campos que demostraban la presencia del secado por Verticillium en Dakota del Norte. Las investigaciones en el invernadero, realizadas en 2004/05, incluyeron 221 variedades, que fueron investigadas a reacción al nuevo linaje de Verticillium. Una variedad de la colección de introducción de plantas, USDA, creada en Rusia, bajo el nombre VNIIMK 8883, que fue inmune, podría servir como fuente ideal de resistencia. Varios híbridos oleaginosos comerciales de los EE.UU., Argentina y Europa, tenían valores de 0.5 ó más bajos en la escala de 0 a 5. Relacionado con ello, un otro hongo, *Phialophora asteris* f. sp. *helianthi*, fue aislado en las plantas de girasol oleaginoso, que demostraban los síntomas de marchitez de follaje en Minota, Dakota del Norte, en el año 2002, para los cuales fue supuesto que eran causados por el hongo *Verticillium dahliae*. Con ocasión de la infección artificial en el invernadero, *Phialophora* produjo los síntomas de marchitez en las líneas de girasol con y sin gen V-1, que eran muy parecidos a los síntomas provocados por *V. dahliae*. Será necesaria una válida identificación de este patógeno, con las plantas que demuestran síntomas de la enfermedad, para evitar malentendimientos en cuanto a estos dos patógenos.

NOUVELLE SOUCHE DE *Verticillium dahliae* EN AMÉRIQUE DU NORD

RÉSUMÉ

Une nouvelle souche de *Verticillium dahliae*, champignon cause du flétrissement et de la pourriture marbrée de la feuille, a été identifiée dans le nord-ouest du Minnesota en 2002. La nouvelle souche ou biotype est caractérisée par son aptitude à vaincre le seul gène dominant de résistance V-1 inclus dans les graines oléagineuses et les hybrides industriels. Des échantil-

lons recueillis en 2003 sur des plantes malades ont confirmé que ce nouveau biotype existait aussi dans la province du Manitoba, au Canada. Une recherche restreinte faite en septembre 2004 a identifié la nouvelle souche dans environ un quart des champs du Dakota du Nord où le flétrissement dû à la verticilliose était présent. Dans des expériences effectuées en serres en 2004/5, nous avons examiné la réaction de 221 cultivars à la nouvelle souche de *Verticillium*. Un cultivar de la collection d'introduction des plantes USDA, développé en Russie sous le nom de VNIIMK 8883, est apparu comme étant immunisé et serait la source idéale de résistance. Quelques hybrides commerciaux à graine oléagineuse des Etats-Unis, d'Argentine et d'Europe ont été évalués à 0.5 ou moins sur une échelle de 0 à 5.

Ayant trait à cela, un autre champignon, le *Phialophora asteris* F. sp. *helianthi*, a été isolé sur des plants de tournesol à graine oléagineuse qui montraient des symptômes de pourriture marbrée de la feuille à Minot, Dakota du Nord en 2002 et on présume qu'ils étaient dus au *Verticillium dahliae*. Dans les inoculations en serre, le *Phialophora* a produit des symptômes de pourriture marbrée sur les lignées de tournesol avec ou sans gène V-1, symptômes qui étaient très semblables à ceux dus au *V. dahliae*. Une identification appropriée de ce pathogène sur des plantes montrant des symptômes de pourriture marbrée sera nécessaire afin que soit évitée la confusion entre les deux pathogènes.