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Formation of microsclerotia of Verticillium dahliae in petioles of infected ash trees

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Abstract

Under young ash trees infected with *Verticillium dahliae*, over 10% of the petioles of fallen leaves were colonized by the pathogen. Counts of microsclerotia in six petioles yielded an average number of 1500 per cm of petiole. Windblown leaves from infected trees very probably contribute to dissemination of the pathogen in forest stands and nurseries.

In the seventies, a new disease problem has arisen with common ash, *Fraxinus excelsior* L., in the Netherlands. The symptoms are wilt, early loss of leaves and dieback or complete death (Hiemstra et al., 1990). A recent study on the etiology revealed that the disease is caused by *Verticillium dahliae* Kleb. (Hiemstra, 1992). The trees become infected via the roots by microsclerotia in soil.

The pathogen has a wide range of host plants. In many of these hosts, in particular leguminous crops (Hoekstra, 1989), cotton (Schnathorst, 1981) and potato (Isaac and Harrison, 1968), microsclerotia are formed in and on the stems of senescent plants. A few hosts are known where microsclerotia are formed in leaves. It has been reported for at least one herbaceous host plant, viz. cotton (Benken and Khakimov, 1964; Wilhelm and Taylor, 1965), and two woody hosts, viz. maple (Zimm, 1918; Townsend et al., 1990) and olive (Tjamos and Tsougriani, 1990). Wilhelm and Taylor (1965) suggested that olive groves became infected by inoculum from cotton fields nearby.

The present study was part of a research on the epidemiology of ash wilt disease. It was investigated whether *V. dahliae* invades the leaves and whether sclerotia are formed. If so, infected leaves might play a major role in the epidemiology of the disease.

In October 1990, leaves of two healthy and two diseased ash trees (five years old) were examined for presence of *V. dahliae*. The diseased trees were inoculated with *V. dahliae* in June 1990 by inserting a drop of a conidial suspension into a cut wound low on their stem. These trees had developed serious symptoms of ash wilt during the summer of 1990. Per tree, 100 petioles of recently abscised leaves were collected within 50 cm from the stem base. Fifty petioles were immediately cleared for examination on microsclerotia. The other ones were buried in a *Verticillium*-free clay soil and stored at 15 °C for 4 weeks and than cleared for examination. For detection of microsclerotia,

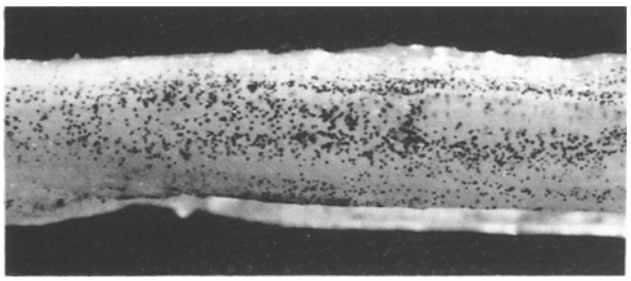

Fig. 1. Microsclerotia of *V. dahliae* in petiole tissue of a fallen ash leaf.

the petioles were cleared by using a modification of the procedure of Kormanik et al. (1980) including the following steps:

1. soak samples in 10% KOH solution at 90 °C for c. 2 h;
2. rinse with three changes of tap water;
3. transfer samples to an alkaline H_2O_2 solution at 20 °C until bleached (for c. 1 h);
4. rinse with three changes of tap water;
5. transfer samples to a formalin – acetic acid – alcohol (FAA) solution (1 : 1 : 18, v/v) and store until examination.

The alkaline H_2O_2 solution (600 ml) was prepared by adding 3 ml of NH_4OH to 30 ml of 10% H_2O_2 and 567 ml water.

The cleared petioles were examined for presence of microsclerotia with a dissecting microscope at 12- and 25-fold magnification.

A small part of each petiole in the first group was used for assessing its colonization by *V. dahliae* by plating sections on a selective medium. At 1 cm from the base of each petiole a segment of 2 – 3 cm length was taken and sterilized (1 minute in 1% NaClO). Two sections (2 mm thick) from the middle of the segment were plated onto the pectate agar medium of Huisman and Ashworth (1974) and incubated at 24 °C for 2 weeks.

With the clearing technique, the microsclerotia could easily be observed (Fig. 1). They were present in a number of petioles of leaves that had been collected under infected trees and never in those of leaves collected around the stem of healthy trees (Table 1). Not all petioles from which the pathogen was isolated had microsclerotia.

Table 1. Number of petioles with microsclerotia and number of petioles from which *V. dahliae* was isolated.

Tree number	Not incubated in soil (n = 50)		Incubated in soil (n = 50)
	Verticillium isolated	with microsclerotia	with microsclerotia
1 (inoculated)	4	3	8
2 (inoculated)	7	1	6
3 (healthy)	0	0	– [a]
4 (healthy)	0	0	0

[a] Treatment not included.

Table 2. Location of microsclerotia of *V. dahliae* in cleared petioles of ash.

Number of petiole	Distance from base of petiole (cm) 0 2 4 6 8 10 12 14 16 18 20 22 24 26
1	+----------+ + + + + + + + + + + + +]a
2	----------+ + + + + + ----------]
3	----------+ + + ----------]
4	+ + + + + + + + + + + ----------]
5	----------+ + + + + + + + ------- + + +]
6	--------+ + + + + + + + + + + + ----------]
7	------+ ----------]
8	--------+ ---------- + ----------+ + ----------]

[a] + and - = with and without microsclerotia;] = top of petiole.

This might be due to suboptimal moisture conditions for their formation. Brinkerhoff (1969) found that high moisture content of the soil strongly promoted formation of microsclerotia in abscised cotton leaves. In our experiment 14% of the incubated petioles contained microsclerotia (Table 1). This is in the same range as Tjamos and Tsougriani (1990) found with petioles from *Verticillium*-infected olive trees incubated under natural conditions.

Counts of microsclerotia in six petioles yielded an average number of about 1500 per cm of the petiole. They were not equally spread through the entire petiole (Table 2). Under the dissecting microscope it could be seen that the microsclerotia were present in all layers of the petiole except the pith. This concurs with the findings of Benken and Khakimov (1964), who reported the same for microsclerotia in petioles of cotton.

In conclusion, *V. dahliae* can invade the petioles of infected ash trees and form microsclerotia. We presume that windblow of dry leaves fallen from infected trees contributes to dissemination of the pathogen in forest stands and nurseries.

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