INFLUENCE OF ROOT-KNOT NEMATODES ON VERTICILLIUM WILT OF MAPLES

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Abstract. Young plants grown from rooted cuttings of four Norway maple cultivars and three red maple cultivars, as well as seedlings of six maple species, were inoculated with combinations of *Verticillium dahliae* and several root-knot nematodes (*Meloidogyne* spp.). The Verticillium-susceptible Norway maples ('Crimson King' and 'Greenlace') were resistant to the nematodes but some plants were killed by the wilt fungus. Of the wilt-tolerant cultivars, 'Parkway' was also tolerant or resistant to the nematodes and the plants did not exhibit wilt symptoms. However, the death of several plants of the supposed wilt-tolerant 'Jade Green', which was susceptible to some of the nematodes, indicated a synergism between the two pathogens. The red maple cultivars were highly susceptible to most of the nematodes, but they did not wilt. Seedlings of Norway maple also showed evidence of the synergistic effect of the fungus and the nematodes.

Species of *Fusarium* and *Verticillium* are important fungal wilt pathogens of both herbaceous crops and woody plants. Since these fungi normally occur in the soil, many studies have sought to determine the potential synergism between a particular fungus and various nematodes (8). Disease complexes of *Fusarium* and root-knot nematodes (*Meloidogyne* spp.), in which the combined effects of both pathogens are significantly greater than those of each pathogen alone, are known in many crop plants (8). In woody plants, McArdle and Santamour (6) used root-knot-*Fusarium* combinations to screen seedlings of *Albizia julibrissin* for resistance to mimosa wilt. Whereas fungal inoculation alone killed about 50% of seedlings derived from parent trees selected for wilt resistance, fewer than 1.5% of such seedlings survived for two years following inoculation with the with the wilt fungus and two species of root-knot nematodes.

The nematodes involved in disease complexes of herbaceous plants with *Verticillium* fungi are mostly migratory types such as the lesion nematode (*Pratylenchus* spp.). Dwinell and Sinclair (1) have also suggested that *P. penetrans* may interact with *V. dahliae* on elm and maple. Root-knot nematodes have been noted to interact with *Verticillium* in tomato (7) and potato(5), but no similar studies have been reported on woody plants.

Among landscape trees, the maples (*Acer* spp.) have long been considered as being most susceptible to Verticillium wilt, and 10 species were reported as hosts in the 1957 compendium of Engelhard (1). These were *A. macrophyllum, A. mono, A. negundo , A. nigrum, A. palmatum, A. platanoides, A pseudoplatanus, A. rubrum, A. saccharinum, and A. saccharum.* More recent work (3, 4) has added *A. campestre* and *A. ginnala* to this list.

Norway maple (*A. platanoides*) and red maple (*A. rubrum*) are the two most widely planted maple species in the United States, and Norway maple is considered the most susceptible to Verticillium wilt. However, Townsend *et al.* (11) reported on the variability among Norway maple cultivars in wilt symptom expression following artificial inoculation. 'Crimson King' and 'Greenlace' were the most susceptible cultivars and 'Jade Glen' and 'Parkway' were the most tolerant. Townsend and Hock (10) also reported variation in disease tolerance among half-sib families of red maple. Most of the trees in these two studies had been inoculated by stem wounding, but some of the Norway maple cultivars had also been inoculated by placing a Verticillium spore suspension into the growing medium of container-grown plants.

Hoitink *et al.* (4), using soil inoculation techniques, found no differences in symptom expression among cultivars of these two maple species. Nine Norway maples (including 'Crimson King') were highly susceptible and seven red maples (including 'Autumn flame', 'October Glory' and 'Red Sunset') appeared to be highly tolerant, with no leaf wilt or streaking in the xylem. The plants used in this study had been budded on seedling rootstocks and thus the soil (and root) inoculation

might not have been truly indicative of cultivar performance. Townsend *et al.* (11), however, reported a high degree of correlation between symptom expression of stem-inoculated budded cultivars and of soil (root) inoculation of the same cultivars growing on their own roots.

Inasmuch as we were testing a wide range of maple species for susceptibility to various root-knot nematodes (9), we decided to extend our research to include a pilot study of potential synergism between nematodes and *Verticillium* in several maple species and cultivars. If a strong synergism could be proved, combined inoculations would provide a more effective screening procedure for wilt resistance.

Materials and Methods

Own-rooted plants (about 30 cm tall) of four Norway maple cultivars ('Crimson King', Greenlace', 'Jade Glen', 'Parkway') had been propagated and donated by J. Frank Schmidt & Sons Nurs., Boring, Oregon, in 1985. Some of the plants from this shipment had been used by Townsend *et al.* (11), in their determinations of Verticillium tolerance. Susan E. Bentz, Horticulturist, U.S. National Arboretum, had also attempted (in 1989) a number of interclonal grafts using these plants and some of those individuals on which the grafts had failed were provided for our tests. The cutting back and pruning of these plants resulted in some above-ground growth irregularities, but the plants generally had one or two main leaders.

The red maple cultivars ('Autumn Flame', 'October Glory', 'Red Sunset') were two-year-old rooted cuttings that had not been pruned back to a single stem. Seedlings of *A. macrophyllum, A. negundo, A. platanoides, A. pseudoplatanus, A. saccharum,* and *A. velutinum* were grown from seed collected in 1989 and represented the same progenies utilized in our studies of nematode susceptibility (9).

The inoculum for all of the nematodes was prepared from root-galls on a susceptible *Salix alba* clone (NA44016) growing in containers in the greenhouse. The inoculum dosage for the larger clonal maples consisted of two 50-ml aliquots of a chopped gall-media mixture, but only one aliquot for the smaller seedlings.

The isolates of *Verticillium dahliae* used for inoculation were supplied by Susan E. Bentz. One isolate, from eggplant, had also been used by Townsend *et al.* (11) in mixed inoculations of Norway maples. Two new isolates, from the roots of infected 'Crimson King' and 'Greenlace' maples, also resulted from the experiment. These three isolates were grown on potato-dextrose agar for about 30 days, at which time the 100 mm petri dish was completely covered with the fungus.

The Verticillium inoculum was prepared by blending two plates of the eggplant isolate and one each of the Norway maple isolates in 800 ml water. The larger maples (clones) were inoculated by inserting a chisel (2.5 cm wide) into the growing media to one-half the depth of the container on four sides of each plant and pouring 25 ml of the inoculum into each cut (100 ml per plant). The smaller seedling maples were inoculated with a total of 50 ml inoculum in two chisel cuts into the medium on opposite sides of each plant. This was not considered a high level of inoculation especially since there was no conscious attempt to sever roots before adding the inoculum.

Four plants each of the seven Norway maple and red maple cultivars (listed in Table 1) were inoculated with each of four different nematodes on 18 April 1991. The nematodes were *M. arenaria* -Race 1, *M. arenaria* -Race 2, *M. incognita,* and *M. javanica.* Thus, 16 plants of each cultivar were inoculated with nematodes. On 17 May 1991, two plants of each cultivar-nematode combination were treated with Verticillium and an additional two plants of each cultivar were treated with Verticillium alone. Two plants of each cultivar served as controls with no nematode or Verticillium treatment.

Likewise, only two seedlings of the other maple species were used for each treatment. Inoculations were made with both races of *M. arenaria* and with *M. incognita* on 15 May 1991, and the Verticillium inoculations were made on 27 June 1991.

Results and Discussion

Data on the response of the Norway and red maple clones to inoculation are presented in Table 1. Neither of the wilt-susceptible cultivars of Norway maple ('Crimson King' and 'Greenlace') were

parasitized by any of the nematodes, but 'Crimson King' appeared to be more susceptible to Verticillium than 'Greenlace'. Of the two wilt-tolerant cultivars, 'Jade Glen' proved to be susceptible (galls and egg masses produced) to three of the four nematodes and five of the six plants subjected to these nematodes and the Verticillium fungus exhibited "total wilt." On the other hand, the wilt-tolerant 'Parkway', although galled by two of the nematodes, did not suffer any leaf loss or wilt. Thus, it appeared that although the successful reproduction of root-knot nematodes on Norway maples were not necessary for the expression of wilt symptoms, certain "wilt-tolerant" but nematode-susceptible clones could suffer severe wilt as result of the synergistic action of both pathogens. Interestingly, on three of the five 'Jade Glen' plants that exhibited total wilt, one or more symtomless epicormic branches developed near the base of the plants. Such behavior would indicate a tendency of this cultivar to be able to "compartmentalize" the Verticillium infection.

With the exception of the 'Autumn Flame' - *M. incognita* combination, all three red maple clones were highly susceptible to all of the nematodes. However, none of the red maples exhibited any wilt symptoms (Table 1).

Of the maple seedlings inoculated with Verticillium and root-knot nematodes, only Norway maple showed evidence of a synergistic effect of the two pathogens. One each of the two plants inoculated with *M. arenaria*-Race 1, *M. incognita,* and *M. javanica* were killed by the fungus-nematode combination. It was not possible to determine the level of nematode infestation on the dead root systems of these plants. The potentially high degree of variability in nematode susceptibility among seedlings of the same progeny noted earlier (9), would suggest, however, that the dead seedlings had been more extensively parasitized than those that had survived.

The Oregon maple (*A. macrophyllum*) seedlings inoculated with Verticillium alone were leafless and virtually dead 30 days after treatment. Terminal growth on both of the seedlings that had been inoculated with Verticillium and *M. arenaria* -Race 1 or *M. incognita* was also killed within 30 days, but none of the leaves below the terminal

Table 1. Response of own-rooted Norway and red maple cultivars to inoculation with *Verticillium dahliae* alone and in combination with root-knot nematodes (*Meloidogyne* spp.) [1]

	M. arenaria-1		*M. arenaria-2*		*M. incognita*		*M. javanica*		*Verticillium*
	Wilt Index	Gall Index	Wilt Index	Gall Index	Wilt Index	Gall Index	Wilt Index	Gall Index	Wilt Index
Norway Maple									
'Crimson King'	0/0	0-0	100/100	0-0	100/100	0-0	0/100	0-0	10/100
'Greenlace'	0/50	0-0	30/100	0-0	0/0	0-0	100/100	0-0	0/0
'Jade Glen'	50/100	4-+	0/50	2-0	100/100	3-+	100/100	3-+	0/0
'Parkway'	0/0	4-0	0/0	0-0	0/0	0/0	0/0	2-0	0/0
Red maple									
'Autumn Flame'	0/0	5-+	0/0	5-+	0/0	1-0	0/0	4-+	0/0
'Oct. Glory'	0/0	5-+	0/0	5-+	0/0	5-+	0/0	4-+	0/0
'Red Sunset'	0/0	5-+	0/0	5-+	0/0	5-+	0/0	5-+	0/0

1. Key to table: Wilt Index is percent leaf wilt of two trees.
Gall Index: First figure refers to gall number: 0= no galls; 1= from one to two galls; 2= three to 10 galls; 3 = 11 to 30 galls; 4= 31 to 100 galls; 5= more than 100 galls per root system. (+) indicates presence of viable egg masses, (0)= no egg masses.

area were lost during the next three months. The seedlings inoculated with Verticillium and *M. arenaria*-Race 2 did not exhibit any wilt symptoms.

No seedlings of the other maple species lost any leaves during the four-month period following Verticillium or Verticillium-nematode inoculation. The seedlings of *A. negundo* and *A. saccharum* were practically symptomless and, while there were minor variations among treatments in *A. pseudoplatanus* and *A. velutinum*, none of the nematode-inoculated plants exhibited more foliar symptoms than those inoculated only with the Verticillium fungus.

Conclusions

This is the first report of synergism between root-knot nematodes and Verticillium wilt in woody plants. The host range of Verticillium wilt is extensive and we are just beginning to expand our knowledge of root-knot nematodes. At this juncture, such synergism would appear to play a minor role among the many maladies of urban and landscape trees, but the door is now open for more detailed studies on a wider range of woody plants.

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Résumé. De jeunes plants issus de boutures racinaires de quatre cultivars d'érable de Norvège et de trois cultivars d'érable rouge, tout comme des semis de six espèces d'érable, furent inoculés aves des combinaisons de *Verticullium dahlia* et de divers nématodes de racines (*Meloidogyne* spp.). Les érables de Norvège susceptibles au *Verticillium* ('Crimson King' et 'Greenlace') étaient résistants aux nématodes mais quelques plants furent tués par le champignon de la flétrissure. Des cultivars tolérants à la flétrissure, le 'Parkway' était aussi tolérant ou résistant aux nématodes et les plants exhibaient aucun symptôme de flétrissure. Toutefois, la mortalité de plusieurs plants de 'Jade Glen', soi-disant tolérants à la flétrissure, mais qui étaient susceptibles à certains nématodes, indique une synergie entre les deux pathogènes. Les cultivars d'érable rouge étaient hautement susceptibles à la majorité des nématodes, mais ils n'étaient pas affectés par la flétrissure. Les semis d'érable de Norvège montraient eux aussi des signes de l'effet synergique entre le champignon et les nématodes.

Zusammenfassung. Junge Pflanzen, die aus eingewurzelten Ablegern von vier Spitz-Ahorn- und drei Rot-Ahorn-Bäumen gewachsen sind, wurden mit Kombinationen von *Verticillium dahlia* und mehreren Wurzelnematoden (*Meloidogyne* spp.) beimpft. Die für *Verticillium* anfälligen Ahornbäume ('Crimson King' und 'Greenlace') waren gegen die Nematoden resistent, aber einige Pflanzen starben durch den Welkenpilz. Von den welketoleranten Kulturen war 'Parkway' auch tolerant bis resistent gegenüber den Nematoden und die Pflanzen zeigten keine Welksymptome. Das Sterben einiger Pflanzen der angeblichen welketoleranten 'Jade Glen', die anfällig für einige der Nematoden war, deutete jedoch eine Synergie der beiden Krandheitserreger an. Die Rot-Ahorn-Pflanzen waren im hohen Maße anfällig für die meisten Nematoden, ohne jedoch Welkesymptome zu entwickeln. Spitz-Ahorn-Sämlinge zeigten ebenfalls Anzeichen der synergistischen Wirkung des Pilzes und der Nematoden.