Discula campestris Infection of Sugar Maple Leaves Associated with Pear Thrips Injury

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ABSTRACT

Nash, B. L., Stanosz, G. R., Taylor, G., and Davis, D. D. 1994. *Discula campestris* infection of sugar maple leaves associated with pear thrips injury. Plant Dis. 78:285-289.

Acervuli of *Discula campestris* were frequently observed on necrotic portions of thrips-injured sugar maple leaves in 1989. Necrosis was present on 82-98% of leaves collected at three sites in May, June, and August 1990 in Potter County, Pennsylvania, but symptoms were not severe. Evaluation of necrotic leaves indicated that 82-91% of the leaves were affected on ≤5% of their surface area. Marginal and interveinal necrosis were most common, while veinal necrosis was rare. The study was expanded to five central Pennsylvania counties in 1991. The median incidence and severity of all forms of necrosis were 0.1-1.0%. Twenty-one of 25 trees were rated positive for *D. campestris* following incubation of symptomatic or asymptomatic leaves in a moist chamber. The fungus was identified on leaves from seven northeastern states: Maine, Massachusetts, New Hampshire, New York, Pennsylvania, Rhode Island, and Vermont.

Sugar maple (*Acer saccharum* Marsh.) is an important ecological and commercial component of northern hardwood forests in Pennsylvania and the northeastern United States. During the last 10 yr, damage to sugar maple foliage has been attributed to feeding and ovipositing of pear thrips (*Taeniothrips inconsequens* (Uzel)). Symptoms associated with pear thrips injury include reduced leaf size, chlorosis/mottling, anthocyanescence, "crinkle," tatter, and necrosis (1-3). Considerable variation in symptom type and severity has been observed at the leaf, tree, and stand level during an infestation. Adult thrips overwinter in the soil, emerge in early spring, feed on flower and leaf buds, and lay eggs under the epidermis of flower and leaf petioles. Larvae hatch from the eggs and continue to feed on young leaves until late May or early June. At that time they enter the soil, where they remain until the following spring (1-3). Because symptoms of so-called thrips injury are reported to intensify as leaves mature, field evaluation of damage and aerial sketch-mapping are often delayed until midsummer. However, since this injury appears to intensify after the thrips have entered the soil, it is possible that other agents, such as foliar fungi, also intensify symptoms during midsummer.

In 1989, acervuli of a fungus were observed on sugar maple foliage collected from several locations in Pennsylvania. These fruiting bodies occurred on water-soaked and necrotic portions of leaves exhibiting symptoms attributed to pear thrips. Acervuli and spores appeared identical to those produced by *Discula campestris* (Pass.) von Arx on young sugar maple seedlings in pear thrips-infested stands in Pennsylvania (9). *D. campestris* is recognized as a cause of anthracnose on sugar maple in Canada (5). The objectives of these studies were to establish the association of *D. campestris* and necrosis on thrips-injured leaves and to investigate the regional occurrence of this anthracnose fungus. Preliminary findings were reported by Nash et al (7).

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Accepted for publication 7 October 1993.

MATERIALS AND METHODS

Potter County survey, 1990. Three study sites were selected in the Susquehannock State Forest in Potter County, Pennsylvania. Each had a significant sugar maple component and a history of recurrent pear thrips infestations. Preliminary visits confirmed current pear thrips activity and damage. At each study site, five dominant or codominant sugar maples were selected for crown sampling in May (during leaf expansion), June, and August. On each site, the outer 1 m of a branch from each cardinal direction was collected by a climber positioned within the crown. The four branches from each tree, subsequently referred to as a tree sample, were placed in a single polyethylene bag, transported to the laboratory, and stored at 2 C. Within 2 days, 80 leaves were arbitrarily selected from each tree sample and placed in a botanical press for 2-3 days. Leaves were removed from the press and placed in labeled manila envelopes for later evaluation. Each of the 1,200 leaves (three sites × five trees per site × 80 leaves per tree) were evaluated for the incidence and severity of the following symptoms: marginal necrosis, interveinal necrosis, veinal necrosis, total necrosis, chlorosis, anthocyanescence, crinkle, and tatter. The last four symptoms were included to document the relationship between necrosis and symptoms associated with thrips injury. Incidence was the number of leaves on which the symptom was observed. Severity was defined as the percentage of leaf surface area with symptoms and was estimated using a modified Horsfall-Barratt scale (4). The upper limits for each of the 11 classes (0 through 10) in this scale were, respectively: 0, 1, 5, 10, 25, 50, 75, 90, 95, 99, and 100%. The Foliar Injury Assessment Module from the Forest-Health Expert Advisory System (6) was used to provide quality assurance/quality control information on disease assessment.

On the day following field collection, 20 additional leaves were arbitrarily selected from each of the tree samples. These 20 leaves were divided into two subsets of 10 leaves, and each subset was placed in a moist chamber. Thus, a total of 300 leaves per collection date (three sites × five trees per site × two chambers per tree × 10 leaves per chamber) were incubated. After 6 days, each leaf was examined under a dissecting microscope for the presence of *D. campestris* acervuli. One leaf from each incubation chamber was selected, on the basis of abundant acervuli, for isolation of the fungus. Fruiting bodies or masses of spores were removed from the leaf surface and transferred to microscope slides. Each specimen was stained and examined for the presence of *D. campestris* spores. Lastly, spores were streaked onto sterile dishes of potato-dextrose agar and incubated at 21 C with a 16-hr photoperiod. Both cultural morphology and characteristics of spores produced in culture were used to confirm the identity of the fungus (11).

Pennsylvania survey, 1991. Five field sites in five counties were selected in 1991 to investigate whether the anthracnose/

thrips damage was more generally distributed in Pennsylvania. The sites, established prior to leaf emergence, were Cherry Springs, Susquehannock State Forest, Potter County (the same location sampled in 1990); Bloss Mountain, Tioga State Forest, Tioga County; Allegheny National Forest, Elk County; Colerain Park, Rothrock State Forest, Huntingdon County; and Blain, Tuscarora State Forest, Perry County (Fig. 1). Sugar maple trees at the Cherry Springs and Bloss Mountain sites had been repeatedly injured by pear thrips and possibly sugar maple anthracnose (G. Laudermilch, *personal communication*). Pear thrips injury was either unreported or described as low at the remaining three sites.

Because both pear thrips and *D. campestris* had been observed on the foliage of understory trees in all three 1990 study sites, pole pruners were used in 1991, rather than the more expensive and time-consuming method using tree climbers. Five understory sugar maples, each adjacent to a dominant or codominant maple, were selected per site. Branch samples approximately 1 m long were collected from at least two sides of each tree. The samples from each tree were bagged, transported to the laboratory, and stored at 2 C.

All branches from a single tree were placed on a laboratory bench and evaluated together as a tree sample. For each tree collection, leaves were examined and the average incidence and severity of marginal, interveinal, and total necrosis were estimated. All samples were evaluated within 2 days of collection. To confirm the presence of *D. campestris*, 20 leaves per sample were arbitrarily selected, incubated, and examined as previously described.

Northeastern states surveys, 1990 and 1991. In 1990, the Environmental Protection Agency conducted a study in New England to evaluate sampling techniques for EMAP-Forests (Environmental Monitoring and Assessment Program—Forests). EMAP field crews visited 17 plots in four states: Connecticut, Maine, New Hampshire, and Vermont. Because destructive sampling was not permitted on plot trees, four dominant or codominant sugar maples were selected just beyond the boundaries of each plot. Foliage from the outer crowns of the 86 trees was collected by tree climbers and evaluated for biotic and abiotic injury by field personnel (10). These samples were then packed in ice chests and sent by overnight mail to The Pennsylvania State University for symptom verification. Necrotic leaves were incubated and examined as described above.

In 1991, state pest specialists from Maine, Vermont, New Hampshire, Massachusetts, Rhode Island, Connecticut, and New York were invited to submit injured sugar maple leaves for isolation of *D. campestris*. Sample information labels were glued to the exterior of self-sealing plastic bags containing several sheets of paper towel. These bags were then placed inside prepaid, preaddressed mailers and sent to state cooperators. State personnel were asked to moisten the paper towels, insert the sample leaves, seal the plastic bag, and return the unit to The Pennsylvania State University in the mailer provided. Upon arrival, the samples were kept in the plastic bags to incubate. After several days, the leaves were examined under a dissecting microscope for necrotic areas and the presence of acervuli of *D. campestris*. The total number of leaves in the sample and the total number of leaves with acervuli were recorded. Spore masses from acervuli were placed on microscope slides, stained, and examined. Spores were also streaked onto sterile plates of potato-dextrose agar and incubated at room temperature. Cultures and spore morphology were used to confirm the identification of *D. campestris*.

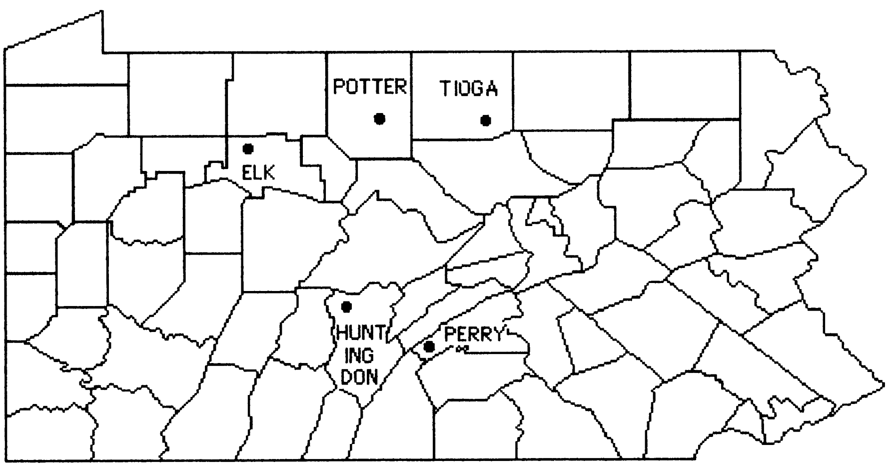

Fig. 1. Sites of the Pennsylvania sugar maple anthracnose survey: Potter County = Cherry Springs, Susquehannock State Forest, 1990 and 1991; Tioga County = Bloss Mountain, Tioga State Forest, 1991; Elk County = Allegheny National Forest, 1991; Huntingdon County = Colerain Park, Rothrock State Forest, 1991; and Perry County = Blain, Tuscarora State Forest, 1991.

RESULTS

Potter County survey, 1990. Pear thrips were present at damaging levels at the three study sites. Larvae and adults were collected on the overstory foliage, and thrips-related symptoms were observed. Chlorosis, marginal necrosis, interveinal necrosis, and total necrosis were the most commonly observed symptoms throughout the growing season (Table 1). Leaf crinkle and leaf tatter were observed at significant levels beginning with the June collection. Anthocyanescence was noted on the second flush of leaves produced in response to early-season defoliation.

Some form of necrosis was identified on 92–95% of the 3,600 sugar maple leaves examined during the course of this study (Table 1). Necrosis was usually present as marginal (78–89% of the leaves) or interveinal (54–70%). Veinal necrosis was usually absent. Symptom expression varied by site and month. For example, incidence levels for all types of necrosis were initially lower at site 10. By August, however, the incidence of interveinal necrosis was higher at site 10 (81%) than at site 2 (60%) or 4 (69%). The percentage of leaves with some form of necrosis (marginal, veinal, or interveinal) ranged from 82 to 98%, depending on the sample month and site.

Although necrosis was very common on sugar maple leaves, only small amounts of leaf tissue generally were involved. Necrotic portions were usually confined to ≤5% of the total leaf surface during all three sampling periods and at all three sites (Table 2). The median severity class for the different types of necrosis was either class 1 (0.1–1% surface area affected) or class 2 (1–5%) in May, although values as high as class 9 (95–99%) were recorded.

The margins of leaves examined in May were generally intact. Although the incidence of marginal necrosis remained approximately constant from May to June (78 and 80%, respectively), the median severity of the symptom decreased from class 2 at sites 2 and 4 in May to class 1 in June. Symptom severity remained constant (class 1) at site 10.

Table 1. Percentage of sugar maple leaves with selected symptoms in 1990[a]

Symptom	Collection month		
	May	June	August
Chlorosis	98	91	93
Marginal necrosis	78	80	89
Veinal necrosis	1	0	1
Interveinal necrosis	54	58	70
Any necrosis	92	92	95
Anthocyanescence	15	5	7
Crinkle	2	51	82
Tatter	0	12	25

[a] Percentage of leaves on which symptom was observed, based on 1,200 leaves per month. Data combined from three sites in the Susquehannock State Forest, Pennsylvania.

Leaves collected in June were typified by ragged margins with a very thin band of necrotic tissue along the margin. Apparently, much of the necrotic tissue detached and fell.

The concurrence of necrosis and acervuli of *D. campestris* varied by site and sample date. Overall, at least one leaf with the fruiting structures was identified on every tree for each sampling date (Table 3). In May, acervuli were identified on at least 65% of the leaves from 14 of the 15 trees. On 11 of these trees, at least 90% of leaves had fruiting bodies. The June levels decreased at all three sites; however, acervuli were identified on approximately 40% of the leaves. The number of leaves with acervuli continued to decline at sites 4 and 10 in August but increased at site 2. In general, leaves with acervuli were most common in May; however, fruiting structures were present on at least one-third of the sugar maple leaves throughout the growing season.

Incubated leaves with abundant acervuli were used to isolate the anthracnose fungus. Approximately 50 isolations per site (five trees per site × two leaves per tree × five isolations per leaf) were completed from the leaves collected in May. The percentage of successful isolations from the three sites ranged from 85 to 91% (Table 4). Approximately 10 isolations were attempted from leaves collected in June, but the acervuli did not rehydrate and *D. campestris* was never recovered. The fungus was again isolated from the August collection, although less successfully (59–67%) than in May.

Pennsylvania survey, 1991. The median incidence class for marginal necrosis, interveinal necrosis, and total necrosis was class 1 (0.1-1% leaves symptomatic) for all five sites. Furthermore, the median severity class for symptomatic leaves was also class 1 (0.1–1% surface area affected). Symptom incidence and symptom severity values never exceeded class 1 for any symptom at any site in this survey.

Although the foliar samples collected from our ground survey (pole pruning) seldom had necrotic symptoms, they were placed in moist chambers and incubated in the same manner as were the highly symptomatic 1990 leaves. Evaluation of spores and pure cultures obtained from these incubated leaves confirmed that *D. campestris* was present in 21 of the 25 leaf collections (one collection per tree × five trees per plot). Furthermore, samples from at least three of five trees in each plot yielded cultures of *D. campestris.*

Northeastern states survey, 1990 and 1991. The leaves sent to The Pennsylvania State University in 1990 by the EMAP field crews were generally

Table 2. Percentage of overstory leaves with ≤5% of surface area necrotic collected from sugar maples infested with pear thrips in the Susquehannock State Forest, Pennsylvania, 1990[a]

	Collection month		
Stand	May	June	August
2	82	86	66
4	84	92	88
10	80	96	95
Av.	82	91	83

[a]Percentage of leaves per site per collection date with ≤5% of the leaf surface area necrotic. The numbers of necrotic leaves for sites 2, 4, 10, and all three combined were, respectively: May, 388, 392, 328, and 1,108; June, 354, 374, 378, and 1,106; and August, 363, 392, 384, and 1,139.

Table 3. Incidence of *Discula campestris* acervuli on incubated sugar maple leaves collected from thrips-infested trees, 1990[a]

Stand Tree	Collection month May	June	August
2			
A	100	75	85
B	95	10	100
C	90	25	20
D	100	38	50
E	100	45	40
Av.	97	39	59
4			
A	90	39	40
B	100	45	15
C	100	35	35
D	95	55	20
E	80	17	10
Av.	93	38	24
10			
A	5	15	50
B	95	80	60
C	65	35	10
D	100	60	10
E	70	30	45
Av.	67	44	35

[a]Percentage of leaves with at least one acervulus of *D. campestris*, based on 20 leaves per tree per month, except trees 2B (based on 10 leaves), 2D (eight leaves), 4A (18 leaves), and 4D (eight leaves).

Table 4. Percentage of successful isolations of *Discula campestris* from incubated sugar maple leaves, 1990[a]

	Collection month		
Stand	May	June	August
2	85	0	59
4	85	0	67
10	91	0	67
Av.	87	0	64

[a]Percentage of isolations yielding *D. campestris*, based on approximately 50 isolations (five trees × two leaves per tree × five isolations per leaf) attempted per site for May and August. Acervuli on June-collected leaves did not rehydrate; approximately 30 isolations were attempted.

Table 5. Incidence of *Discula campestris* on leaves collected from thrips-infested sugar maples in four northeastern states, 1991[a]

State Location	No. of leaves sampled	Percent leaves with *Discula*[b]	Presence of *Discula*[c] Spores	Culture
Massachusetts				
Wendling farm	35	63	+	+
	26	31	+	+
	18	39	+	+
Krug farm	70	43	+	+
	36	14	+	+
	31	61	+	+
New York				
Beebe State Forest	15[d]	13[d]	+	+
	15[d]	40[d]	+	+
B. Brown farm	6[d]	100[d]	+	+
Cattaraugus County	38	10	+	+
Willow Bend farm	65	62	−	+
Heiberg Memorial Forest	78	21	−	+
Arnot Forest	99	0	−	−
Allegheny State Park, site 1	>100[d]	0[d]	−	−
John Gibbons farm	81	23	−	+
Rhode Island				
Providence Water	45	95	+	+
Route 102, Exeter	19	79	+	+
Vermont				
Northfield, tree 1	36	100	+	+
tree 2	25	84	+	+
Motyka, Northfield	50	30	+	+
Dorset	105	0	+	−
Total	993	31		

[a]Various state agencies collected leaves and mailed them to The Pennsylvania State University for examination to detect *D. campestris*.
[b]Based on presence of at least one fruiting body after incubation in a moist chamber.
[c]Spores: recovered and confirmed under microscopic evaluation; + = spores on at least one leaf, − = no spores recovered. Culture: + = *D. campestris* isolated from at least one leaf, − = *D. campestris* not isolated from sample.
[d]Number of leaves estimated (some discarded because of contamination).

healthy. The median percent surface area injured (all biotic symptoms) was the 0.1–1% class. The fungus was successfully isolated from only one of 16 trees in New Hampshire, five of 20 trees (from three different plots) in Vermont, and one of 28 trees in Maine. The fungus was not recovered from any of the four sugar maples sampled in Connecticut.

In 1991, state pest specialists from four states sent a total of 21 leaf collections to establish the presence or absence of *D. campestris* (Table 5). The number of leaves per collection ranged from six to >100. In some cases, leaves were partially decomposed and were discarded. Because the number of discarded leaves was not recorded, the number of leaves in four collections was estimated. On the basis of presence of acervuli and spores on incubated leaves, 308 (31%) of 993 leaves were rated positive for *D. campestris* (Table 5). The fungus was confirmed from all four states that participated in the survey (Massachusetts, New York, Rhode Island, and Vermont). These results reveal that the fungus is present in at least seven northeastern states: Maine, Massachusetts, New Hampshire, New York, Pennsylvania, Rhode Island, and Vermont.

DISCUSSION

D. campestris was associated with necrosis commonly attributed to thrips injury in 1990. However, whether the fungus was the cause of this necrosis or simply colonized leaf tissue that was injured or dead from thrips infestation is not known. Until this relationship is clarified, researchers and pest managers should be cautious when attributing symptomatic sugar maple foliage to pear thrips alone. Ovipositing and feeding by pear thrips adults, as well as feeding by the larvae, provide many wounds on individual leaves. It has been demonstrated that mechanical wounds favor development of disease in sugar maple seedlings inoculated with a *D. campestris* isolate identical to the fungus observed in this study (9). Therefore, the high incidence of necrotic leaf tissue observed in this study may have resulted from an interaction between the anthracnose fungus and pear thrips.

Estimating necrosis by the part of the leaf blade affected showed that the dead tissue was usually marginal or interveinal rather than veinal. Apparently, much of this necrotic tissue detached and fell. Some of the difficulty in isolating the fungus from leaves collected in June might be explained by the loss of fungal fruiting structures as necrotic tissue was removed. Furthermore, those few acervuli still present on the leaf surface would not rehydrate even after 8 days of incubation in moist chambers. Spore masses, common on leaves collected in May, were not detected on leaves collected in June. The greater incidence of the fungus in August may have been due to secondary infections on young leaves initiated in June in response to prior thrips injury in May and early June.

The effect of anthracnose-induced tissue necrosis is difficult to estimate. Although frequent, the small amount of dead tissue suggests minimal effect on tree health. However, because necrotic tissue often dropped from affected leaves, the actual cumulative tissue loss would be greater than that observed at any one time. Leaf deformities resulting from necrosis of small portions of the marginal meristematic tissue might detrimentally affect leaf and crown productivity and, ultimately, tree growth and vigor.

The colonization of overstory sugar maple leaves by this fungus might have serious consequences for sugar maple seedlings emerging from the forest floor. Recent research (8,9) demonstrated that the fungus is pathogenic on sugar maple seedlings and can cause mortality. Surveys in thrips-infested stands have revealed approximately 90% mortality of sugar maple seedlings and a high frequency of fungus association with these seedlings. Fruiting bodies on overstory leaves are a source of inoculum that could be rain-washed onto developing, thrips-infested seedlings. Subsequent anthracnose development on these seedlings may help to explain the demise of sugar maple regeneration in some areas.

Symptoms associated with pear thrips or *D. campestris* were virtually absent throughout Pennsylvania in May 1991. If this study had relied solely on field-collected data, or even on laboratory examination of field-collected leaves, we would have concluded that *D. campestris* was absent from most, if not all, of the sugar maple stands evaluated in central Pennsylvania and the cooperating northeastern states. However, incubation of asymptomatic leaves in moist chambers identified the fungus on 31% of the leaves submitted from the northeastern region. We cannot explain why asymptomatic leaves yielded *D. campestris*, but the fungus may survive as an endophyte or epiphyte. Proper environmental conditions or sufficient wounding by pear thrips may allow the fungus to aggressively colonize leaf tissue. Further controlled studies to determine the relationship between this fungus and pear thrips and to define the environmental conditions necessary for sporulation and infection should be completed.

The potential for year-to-year variability in symptom expression was observed at site 2, which was examined in both 1990 and 1991. In May 1990, most leaves at this site showed chlorosis (98%) and marginal necrosis (97%), and many leaves had interveinal necrosis (64%) and anthocyanescence (11%). At the same phenological time in 1991, few leaves (≤1%) had marginal necrosis, interveinal necrosis, anthocyanescence, tatter, or cupping. Chlorosis was frequently detected (75.1–90% of leaves), but little foliar area was affected (median severity class 2 [1.1–5%]) as compared to 1990 (median severity class 6 [50.1–75%]). These data illustrate that the incidence and severity of symptoms associated with anthracnose and pear thrips can change dramatically in the course of 2 yr at the same site.

This study has shown that *D. campestris* has a regional distribution, is associated with pear thrips–injured foliage, but may be isolated from necrotic and sometimes asymptomatic leaves of sugar maple. The fungus may have little direct impact on overstory foliage of sugar maple but could be an important inoculum source for infection of understory regeneration.

ACKNOWLEDGMENTS

We thank Gary Laudermilch, regional pest specialist with the Pennsylvania Bureau of Forestry, for providing expertise on site selection and thrips biology, and Norma Kenyon, seasonal worker with the Pennsylvania Bureau of Forestry, and Coralie Bloom, for technical assistance. We also thank the Pennsylvania Bureau of Forestry district foresters from the Rothrock, Susquehannock, Tioga, and Tuscarora state forests for their support, and William Jackson and Kelly Riddle, of the USDA Forest Service, Forest Health Protection, Morgantown, West Virginia, for their valuable suggestions and information on sugar maple stands on the Allegheny National Forest. Lastly, we acknowledge the USDA Forest Service, Northeastern Area State and Private Forestry, Forest Health Protection, and the U.S. Environmental Protection Agency Environmental Monitoring and Assessment Program for their financial support (federal grants NA-90-0126 and NA-91-0054).

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