Angular Leaf Scorch, a New Disease of Grapevines in North America Caused by *Pseudopezicula tetraspora*

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ABSTRACT

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Pseudopezicula tetraspora, a recently described genus and species of ascomycete, was shown to be the causal agent of a newly recognized disease, angular leaf scorch of grapevines, in eastern North America. The disease has been found in the Finger Lakes region of central New York and in the Lake Erie region of western New York and Pennsylvania. In September 1986, the percentage of leaves with symptoms of angular leaf scorch in Finger Lakes commercial vineyards of *Vitis* interspecific hybrid DeChaunac, Rougeon, and Ventura was 72, 78, and 78%, respectively. Symptoms of angular leaf scorch were similar to the symptoms of a disease caused by *Pseudopezicula tracheiphila* (= *Pseudopeziza tracheiphila*) in European vineyards and known as rotbrenner. Angular leaf scorch was observed on 18 species of *Vitis*, on interspecific hybrids of *Vitis*, and on *Parthenocissus quinquefolia* (Virginia creeper). Diseased tissue placed in a moist chamber at 20 C developed numerous, small, gelatinous apothecia containing paraphyses and asci with four binucleate, reniform ascospores typical of *P. tetraspora*. Ascospores produced in pure culture were used as inoculum to reproduce the disease in the greenhouse. Fungicide trials and grower experience indicated that mancozeb and benomyl were effective control measures, whereas captan was ineffective.

Since first described in Switzerland by Müller-Thurgau (9), the occurrence of rotbrenner disease has been limited mainly to the cool, grape-growing regions of Europe, although there are also reports from Turkey, Tunisia, and Brazil (1). In European vineyards, the fungus *Pseudopezicula tracheiphila* (Müll.-Thurg.) Korf & Zhuang (= *Pseudopeziza tracheiphila* Müll.-Thurg.) (6) causes a leaf scorch that is generally delimited by major veins. Fruit are not infected directly, but the pedicel and portions of the subrachis may become infected, causing fruit distal to the site of infection to wither and dry. This phase of the disease can cause substantial crop loss (15).

Grapevines with leaf symptoms similar to rotbrenner had been observed occasionally on various grape cultivars in New York vineyards. In 1985, the disease was tentatively identified as rotbrenner, caused by *Pseudopeziza tracheiphila* (10). However, the fungus associated with the disease in New York has subsequently been described as a new genus and species, *Pseudopezicula tetraspora* Korf, Pearson & Zhuang (ATCC 62299) (6). Observations on the disease, now designated "angular leaf scorch," techniques for diagnosis, pathogenicity tests, and preliminary observations on control are described herein. A portion of this work was reported previously (16).

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MATERIALS AND METHODS

Isolation. *P. tetraspora* was isolated from leaf tissue segments (1–2 mm square) excised from the yellow margin between necrotic and green tissue. The tissue segments were soaked in 0.5% sodium hypochlorite for 30 sec and plated directly on 2.5% malt-extract agar medium (Difco Laboratories, Detroit, MI) or on 2.5% malt agar overlaid with leaf pieces of *Vitis riparia* Michx. that had been sterilized in propylene oxide. Petri dishes were incubated at 20 C and provided with a daily 16-hr photoperiod of fluorescent light (General Electric, F40CW.RS.WM, 34W: 127 $\mu E \cdot m^{-2} \cdot s^{-1}$).

Scanning electron microscopy (SEM). Necrotic leaf segments bearing apothecia were fixed in 4% glutaraldehyde buffered with 0.05 M KPO_4 at pH 6.5 for 3 hr. The samples were rinsed six times in 0.05 M KPO_4 at 10-min intervals, rinsed twice in distilled water, and postfixed in 1% OsO_4 for 1.5 hr. The segments were then rinsed in distilled water six times at 10-min intervals, dehydrated in a seven-step acetone series, critical-point dried, mounted, and sputter-coated with gold

before examination and photography.

Transmission electron microscopy (TEM). Apothecia were removed from leaf tissue, fixed, and dehydrated as above. After dehydration, the samples were imbedded in an Epon-Araldite resin, polymerized, and sectioned at 70–100 nm. The sections were stained with aqueous uranyl acetate and lead citrate.

Pathogenicity tests. Isolates of *P. tetraspora* were obtained from diseased grape leaves at Dresden and Geneva, NY. Subcultures were grown on 2.5% malt agar overlaid with sterile pieces of grape leaves. Cultures were incubated at 20 C for 10 days to produce an abundance of apothecia containing mature ascospores. Ascospore suspensions were then prepared by removing apothecia and placing them in sterile distilled water for 30 min to allow discharge of ascospores. The suspension was then mixed on a vortex mixer for 1 min, particulate matter was allowed to settle for 2 min, and the resultant spore suspension was decanted and adjusted to 60,000 spores per milliliter using a hemacytometer.

All leaves of five potted grapevines of the interspecific hybrid Chancellor were inoculated by placing four drops of the ascospore suspension at four locations on the adaxial surface of each leaf. The youngest unfolded leaf at the time of inoculation was marked by loosely tying a piece of string around the petiole. Control vines were inoculated with sterile distilled water. Inoculated plants were placed in a mist chamber at 20 C for 72 hr, then moved to the greenhouse. Symptom type and location were noted daily for the first 7 days after inoculation and then weekly. Six weeks after inoculation, segments of leaves showing typical symptoms of angular leaf scorch were placed on 2.5% malt agar. Fungi growing from these leaf pieces were transferred to malt agar overlaid with grape leaf pieces. The number of cultures producing apothecia of *P. tetraspora* was recorded after 10 days.

RESULTS

Symptoms and diagnosis. Lesions on leaves in the field appeared first as chlorotic spots, which enlarged and progressed from yellow to reddish brown as the tissue became necrotic. The lesions usually retained a yellow margin (red margin in red and black cultivars of *V. vinifera* L.) and were generally delimited by the major veins (Fig. 1). The adaxial surface of necrotic lesions occasionally had a varnishlike sheen, especially in late summer. When lesions coalesced, more than half of the leaf blade was often destroyed.

Tissue was excised from the yellow or red margin between necrotic and healthy zones of diseased leaves. The tissue was cleared by boiling in 2% aqueous KOH for 2–3 min, squashed between a cover glass and microscope slide, and observed with a light microscope. Hyphae in sine wave patterns were observed in vessel elements (Fig. 2).

When excised necrotic leaf segments were incubated on wet filter paper (7) in petri dishes for 3–4 days at 19–20 C under a daily 16-hr photoperiod of fluorescent light, aggregations of hyaline, gelatinous apothecia, 0.1–0.3 mm in diameter, were produced. The apothecia contained paraphyses and 20–80 asci, each with four reniform, binucleate ascospores (11–14.7 × 24.2–32.5 μm) (Fig. 3C and D); rarely, five-spored asci were observed. The nuclear condition was readily observed using phase contrast microscopy (Fig. 3C) or TEM (Fig. 3D).

P. tetraspora was consistently isolated from diseased tissue on 2.5% malt-extract agar. Hyphae on agar grew in coiled and wavy patterns similar to the growth habit in vessel elements of infected tissue (Fig. 2). Within 10–14 days at 20 C, cultures growing on malt-extract agar overlaid with sterile grape leaf segments produced numerous apothecia containing mature ascospores typical of *P. tetraspora*. Germinating ascospores first produced a germination vesicle on one side from which a germ tube emerged (Fig. 3A and B). These germination vesicles appear to be characteristic of the fungus.

Pathogenicity tests. Areas of leaves inoculated with an ascospore suspension developed brown necrotic flecks within 36 hr after inoculation. After 6 days,

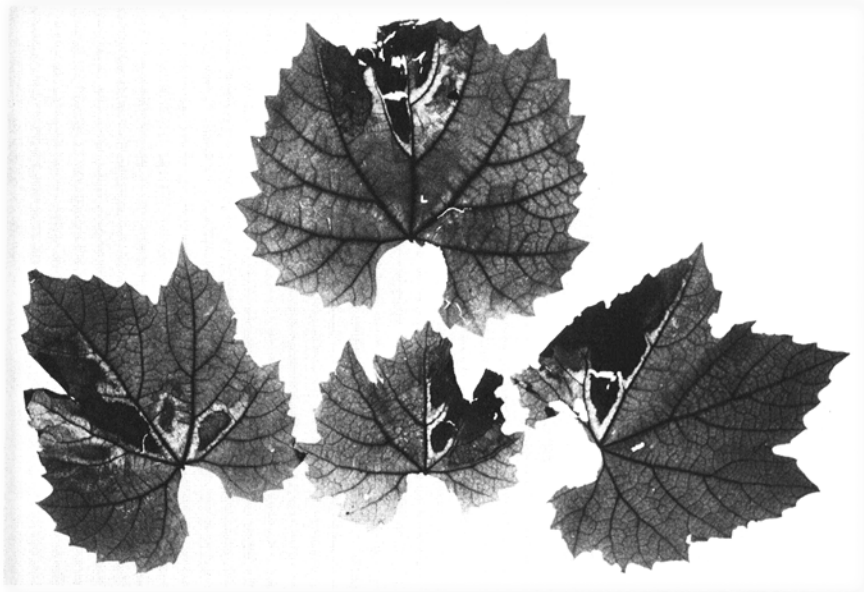

Fig. 1. Angular leaf scorch symptoms showing necrosis delimited by major veins.

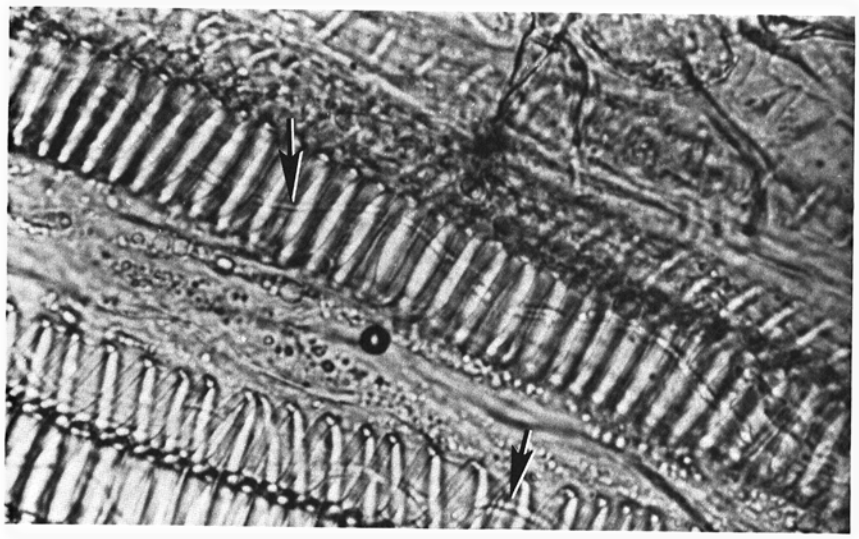

Fig. 2. Hyphae (arrows) of *Pseudopezicula tetraspora* in vessels of Aurore grape leaf. (×553)

necrotic veinlets and yellowed or necrotic interveinal tissue were observed at inoculation sites. Leaves with these initial symptoms developed typical angular leaf scorch symptoms approximately 3 wk later. In addition to the necrotic, yellow-margined, angular lesion typically seen in vineyards, a variety of symptoms were observed on the leaves of inoculated plants, including puckering, shot holes, dimpling, and yellowing. Only three to seven of the youngest leaves on a shoot present at inoculation were susceptible to infection. Isolations from 35 of 36 typically symptomatic leaves yielded *P. tetraspora* (Table 1), thus completing Koch's postulates. Isolations from leaves showing symptoms other than those typical of angular leaf scorch did not yield *P. tetraspora*.

Field observations and cultivar susceptibility in 1986. The most severe outbreak of angular leaf scorch to date was observed during the growing season of 1986, and it was associated with an exceptionally wet period in June and July, 160 and 158 mm rainfall, respectively (Table 2). Leaf litter collected from the floor of an Aurore (interspecific hybrid) vineyard at Dresden, NY, during bud break on 29 April contained apothecia of *P. tetraspora* with mature ascospores. A week of wet weather beginning 19 May apparently provided the appropriate conditions for primary infection, and the first symptoms of angular leaf scorch appeared in mid-June on the six basal leaves of shoots. Further disease development was not observed until early September, when a severe epidemic was apparent in several vineyards. Three commercial vineyards of interspecific hybrid DeChaunac, Ventura, and Rougeon had 72, 78, and 78% leaf infection, respectively, on 9 September. DeChaunac, Ventura, and Rougeon vines in these same vineyards had 19, 13, and 24% missing leaves, respectively, at the basal four or five nodes of shoots. Each of these vineyards was on a seasonal fungicide program of captan plus sulfur.

The high disease incidence in 1986 provided an opportunity to compare the relative susceptibility of *Vitis* species and cultivars under field conditions and the production of apothecia on infected leaves of various species. Foliar lesions were observed on 17 species of *Vitis* at Geneva, NY, and apothecial production was subsequently induced on these leaves in the laboratory. A field rating system of 0–3, where 0 = none (not susceptible), 1 = 1–9 (slightly susceptible), 2 = 10–50 (moderately susceptible), and 3 = >50 (extremely susceptible) infected leaves per vine, was used to determine relative susceptibility of 17 species of *Vitis*. The species were categorized as: slightly susceptible—*V. aestivalis* Michx., *V. argentifolia* Munson, *V. berlandieri* Planch., *V. cordifolia* Michx., *V. labrusca* L., *V. rubra* Michx., *V. rupestris* Scheele, and *V. vinifera*; moderately susceptible—*V. betulifolia* Diels & Gilg., *V. champini* Planch., *V. cinerea* Engelm., *V. longii* Prince, and *V. vulpina* L.; and extremely susceptible—*V. andersonii* Rehd., *V. doaniana* Munson, *V. piasezkii* Maxim., and *V. riparia*. It is interesting to note that two native species of the northeastern United States, *V. aestivalis* and *V. labrusca*, were relatively resistant to *P. tetraspora*, whereas *V. riparia*, a common wild grape in New York, was highly susceptible. There appeared to be no relation between the relative susceptibility of these *Vitis* species and the production of apothecia on diseased necrotic tissue.

Twenty-nine commercial cultivars were evaluated for angular leaf scorch disease severity under field conditions at Geneva, NY. Based on the field rating system of 0–3, cultivars of *V. labrusca*-type were categorized as: slightly susceptible—Catawba, Concord, Delaware, Dutchess, Foch, Fredonia, Ives, and Niagara; and moderately susceptible—Elvira, Missouri Riesling, and Steuben. Interspecific hybrid cultivars were categorized as: slightly susceptible—Himrod, Remaily Seedless, and Vidal blanc; moderately susceptible—Baco noir, Canadice, Cayuga White, Rosette, Seyval, and Vignoles; and extremely susceptible—Aurore, Chancellor, Chelois, DeChaunac, and Rougeon. *V. vinifera* cultivars Gewürztraminer, Pinot noir, and White Riesling were slightly susceptible and Chardonnay was moderately susceptible. The *V. labrusca* types and the *V. vinifera* cultivars were generally less susceptible than the interspecific hybrids.

Parthenocissus quinquefolia (L.) Planch. (Virginia creeper), another member of the Vitaceae, was also susceptible to angular leaf scorch. Infected leaflets of *P. quinquefolia* showed a bright red color followed by a reddish brown necrosis.

The appearance of new symptoms in early September 1986 raised a question regarding the source of inoculum. Because apothecia with mature ascospores are produced in 3–4 days in the laboratory when infected leaves remain wet, we suspected that apothecia might be produced on current-season lesions in the field. In a subsequent vineyard study, shoots of Aurore and Chancellor vines bearing symptomatic leaves were sprayed with water and enclosed in plastic bags. After 1 wk, bagged and nonbagged infected leaves were removed from the vine and examined for apothecial production. None of the nonbagged leaves had produced apothecia, but 41.0 and 41.5% of the bagged Aurore and Chancellor leaves, respectively, had apothecia containing mature ascospores

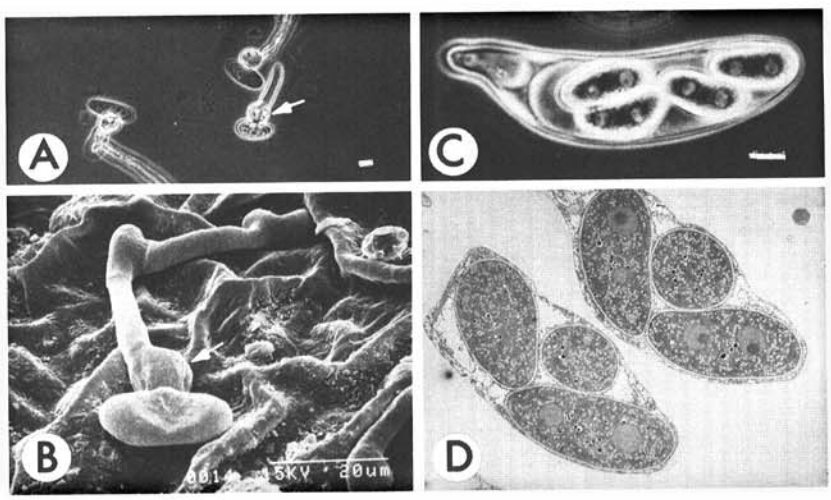

Fig. 3. Ascospores of *Pseudopezicula tetraspora*: **(A)** Germinated ascospores with germination vesicles (arrow) from which germ tube emerges. Scale bar = 10 μm. **(B)** Scanning electron photomicrograph of germinated ascospore with germination vesicle (arrow) on surface of necrotic leaf. **(C)** Ascus containing four ascospores, each with two nuclei, as seen with phase contrast microscopy. Scale bar = 10 μm. **(D)** Thin section of binucleate ascospores. (×2,000).

Table 1. Results of pathogenicity tests on Chancellor grapevines inoculated with ascospore suspensions of *Pseudopezicula tetraspora* in the greenhouse

Vine	Fraction of inoculated leaves with symptoms of angular leaf scorch[a]	Fraction of leaves yielding *P. tetraspora*[b]
1	8/23	8/8
2	9/17	8/8
3	6/32	5/5
4	10/24	8/9
5	10/22	6/6

[a] Number of leaves with symptoms/number of leaves inoculated.
[b] Number of leaves from which *P. tetraspora* was isolated/number of leaves cultured.

on their abaxial surfaces. In a separate study, 92% of infected Chancellor leaves collected from vines in the field on 10 October 1986 had apothecia on their lesions. Similarly, after a prolonged rainy period in September 1987, infected leaves on wild *V. riparia* vines had numerous apothecia of *P. tetraspora*.

Control. In a nonreplicated trial at Watkins Glen, NY, benomyl (Benlate 50WP) at 0.56 kg a.i./ha and mancozeb (Manzate 200 flowable) at 2.24 kg a.i./ha were each applied with an air-blast sprayer to eight rows of an Aurore vineyard. The first application was made on 12 May 1986, when shoots (about 7 cm long) had three unfolded leaves, followed by applications on 23 May and 3 June. No other fungicides were applied until mid-June, when symptoms were first observed. On 9 July, the number of infected leaves per vine observed in 100-vine samples from the nontreated, benomyl-treated, and mancozeb-treated vines averaged 12.6, 4.2, and 6.1, respectively.

In a fungicide trial on Chancellor grapevines conducted at Geneva, NY, in 1986, various formulations of captan, maneb + zinc salt, and mancozeb were compared. The first spray was applied on 28 May following the first presumed angular leaf scorch infection period during the week of 19 May. Subsequent sprays were applied on 10 June, 1 and 15 July, and 5 and 19 August. None of the captan treatments was efficacious, but each of the maneb + zinc salt treatments and the mancozeb treatment provided moderate control of angular leaf scorch (Table 3).

Table 2. Precipitation at Geneva, NY, and relative disease incidence of angular leaf scorch during the growing seasons of 1983–1987

Year	Rainfall (mm)/number of days with measurable rainfall						Disease incidence
	May	June	July	Aug.	Sept.	Total	
1983	76/15	87/8	13/4	74/14	52/10	302/51	Low
1984	144/19	42/7	63/9	170/17	107/12	526/64	Trace
1985	65/13	133/19	44/9	44/8	76/13	362/62	Moderate
1986	67/11	160/15	158/13	63/14	78/11	526/64	High
1987	25/7	120/14	143/13	85/11	130/19	503/64	Low

Table 3. Control of angular leaf scorch on Chancellor grapevines at Geneva, NY, in 1986

Treatment	Active ingredient (formulation) rate/ha[x]	Percent infected leaves[y]
None	...	36.3 ab[z]
Captan (Captan 50WP)	2.24 kg (4.48 kg)	37.2 ab
Captan (Captec 4L)	1.68 kg (3.51 L)	34.0 b
Captan (Captec 4L)	2.25 kg (4.68 L)	41.3 a
10% Metalaxyl + 48% mancozeb (Ridomil MZ 58)	0.22 kg metalaxyl + 1.08 kg mancozeb (2.24 kg)	17.7 c
Maneb + zinc salt (Manex 4L)	2.69 kg (5.61 L) 1.68 kg (3.51 L)*	17.0 cd
Maneb + zinc salt (Dithane M-22 Special 80WP)	2.69 kg (3.36 kg) 1.57 kg (1.96 kg)*	12.4 d

[x] Treatments applied by hooded-boom sprayer at 2,758 kPa and 1,871 L/ha to four four-vine plots arranged in a randomized complete block design on 28 May, 10 June, 1 and 15 July, and 5 and 19 August. * = Rate change after 10 June application.

[y] Data based on a rating of all leaves on 10 shoots randomly selected from the two center vines on 3 September.

[z] Letters indicate groupings of treatments that do not differ significantly ($P \leq 0.05$) as analyzed by Waller-Duncan's exact Bayesian k-ratio LSD rule when applied to arcsin transformed data.

DISCUSSION

The origin of *P. tetraspora* in North America is unknown, although the origin of *P. tracheiphila* is considered European (4). The fact that the fungus causing angular leaf scorch has four ascospores per ascus is but one character that distinguishes it from its European counterpart. Korf et al (6) have elaborated on additional characters. Based upon a different etiologic agent, the new disease designation, angular leaf scorch, is justified. Nevertheless, there is a striking similarity in symptom expression between the European rotbrenner and the American angular leaf scorch diseases. Furthermore, *P. tetraspora*, like *P. tracheiphila* (8,9,13,14), shows a sine wave pattern of hyphal growth in vessel elements of infected tissue.

Although angular leaf scorch symptoms have been observed in New York during the past several years, serious incidences of the disease were not observed before 1985. We do not know if this newly recognized disease is a recent introduction or is endemic but has been partially controlled by existing fungicide programs, or if disease incidences are likely to increase. It does appear, however, that the abundance of highly susceptible wild *V. riparia* will serve as a reservoir for this pathogen in the foreseeable future. In Europe, benomyl (5) and mancozeb (14) appear to be the most effective fungicides for control of rotbrenner. Perhaps the decline in the use of benomyl in New York, because of fungicide resistance in *Uncinula necator* (Schw.) Burr. (12) and *Botrytis cinerea* Pers. (11), is partly responsible for the increased incidence of angular leaf scorch in commercial vineyards.

According to European pathologists, reproduction of rotbrenner disease symptoms by artificial inoculation under controlled conditions is extremely difficult and rarely successful. The difficulties encountered in obtaining infections by *P. tracheiphila* under greenhouse conditions have been discussed at length by Eberhard (2), who found preconditioning of leaves with alcohol treatment beneficial in obtaining infection. However, we did not experience similar difficulties in duplicating angular leaf scorch symptoms under greenhouse conditions.

Symptom development of rotbrenner in Europe has been associated with environmental conditions that induce water stress in the vine (3,9,13,17). Vines inoculated with *P. tetraspora* were watered daily, however, and water stress did not appear to be required to induce symptom expression of angular leaf scorch in greenhouse tests. Futhermore, exceptionally dry weather occurred during the growing seasons of 1983 and 1985, when disease incidence was low and moderate, respectively. In contrast, rainfall was excessive in 1984, 1986, and 1987, when disease occurred in trace, high, and low amounts, respectively. Furthermore, total rainfall for the growing seasons of 1984 and 1986 was identical (526 mm). Rainfall patterns during these 2 yr seems to indicate that precipitation during the month of June is crucial for disease development.

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