Etiology of Stemphylium Leaf Blight of Onion

Nina Shishkoff and James W. Lorbeer

Anna E. Jenkins postdoctoral fellow and professor, respectively, Department of Plant Pathology, New York State College of Agriculture and Life Sciences, Cornell University, Ithaca 14853.

We thank Marvin E. Miller, Dennis A. Johnson, Emory G. Simmons, Dave LoParco, and Douglas Robson for their assistance. Special thanks to Tim Martinson for his help with experimental design and statistical analysis.

Accepted for publication 13 September 1988.

ABSTRACT

Shishkoff, N., and Lorbeer, J. W. 1989. Etiology of Stemphylium leaf blight of onion. Phytopathology 79:301-304.

A species of *Stemphylium* was isolated from lesions on leaves of onion plants grown commercially on organic soil in New York in 1985 and identified as *Stemphylium vesicarium*. In controlled inoculations the fungus caused lesions on leaves of all ages of onion plants, especially on older leaves. Rubbing leaves of greenhouse-grown onion plants with bleached, nonabsorbent cotton to damage the cuticle increased the number of lesions per leaf. The number of lesions per centimeter of leaf length increased the longer plants were incubated in a mist chamber after inoculation with the pathogen. Lesions similar to those produced by the New York isolates were formed on onion leaves inoculated with isolates of *S. vesicarium* from asparagus in Washington and from onion in Texas.

Stemphylium species cause a number of diseases of plants such as black rot of carrot (*Daucus carota* L. var. *sativa* DC.), caused by *S. radicinum* (Meier, Drechsler, Eddy) Neerg.; leaf spot of alfalfa (*Medicago sativa* L.), caused by *S. botryosum* Wallr.; and gray leaf spot of tomato (*Lycopersicon esculentum* Mill.), caused by *S. solani* Weber or *S. floridanum* Hannon and Weber (1,8). These diseases are serious in warm, wet weather and under moist storage conditions. *S. vesicarium* (Wallr.) Simm. has been reported to cause a leaf blight of onion (*Allium cepa* L.) in Texas (6), Wisconsin (11), and India (7), and also causes Stemphylium leaf spot and purple spot of asparagus (*Asparagus officinalis* L.) (2,4,5).

In 1985, a previously unknown leaf blight was observed on onion plants commercially grown on organic soil near Prattsburg, NY. Affected onions had pale, oval lesions that turned brown as they expanded, eventually coalescing and killing leaves. A species of *Stemphylium* was isolated from diseased tissue. In this paper, the *Stemphylium* sp. is identified as *S. vesicarium*, its pathogenicity to onions is confirmed, and some of the conditions conducive to the disease are described.

MATERIALS AND METHODS

The onion plants used in all experiments were the cultivar Downing Yellow Globe, a long day length storage onion. They were either grown from seeds surface sterilized in 0.5% sodium hypochlorite for 5 min or sprouted from surface-sterilized bulbs and grown in the greenhouse at 18 C.

Taxonomy and pathogenicity. Isolates of *S. botryosum* from alfalfa (EGS 04-118) and *S. vesicarium*, including one isolated from lesions on asparagus grown in Washington (Sv[asp]), one from onion in Massachusetts (EGS 24-001), one known to cause lesions on onion grown in Texas (Sv[tex]), and one that was isolated from lesions on onions in Prattsburg (PS-85B), were used in the experiments conducted. To compare conidium dimensions, the isolates EGS 24-001, EGS 04-118, and PS-85B were grown on onion leaf agar (1.5–2 g of autoclaved fresh onion leaves added to 10 ml of molten water agar in a 100-mm petri dish) in an 18 C incubator with a 12-hr photoperiod of near-ultraviolet radiation. Conidia were collected after 2 wk and their length and width at the middle transverse septum was measured, and the length-to-width (l:w) ratio was calculated. The l:w ratios were compared by using the Student-Newman-Keuls test.

The perfect state (*Pleospora*) of most isolates was obtained in 2-to 4-mo-old cultures and ascus size and spore size was measured.

To inoculate leaves of onion plants, conidia from 2- to 3-wk-old cultures were collected by adding a few milliliters of distilled water to each petri dish, dislodging conidia with a fine brush, and aspirating the conidial suspension into a flask containing 10 ml of distilled water with a drop of Tween 20 (polyoxyethelene sorbitan mono-oleate) added to allow greater spore wetting. Spore concentrations were measured with a hemacytometer. The control solution contained 10 ml of distilled water with a drop of Tween 20 added.

To compare the pathogenicity of PS-85B to that of known isolates of *S. vesicarium* and *S. botryosum*, 16 sprouted onion bulbs with 3-wk-old leaves were selected. Most bulbs developed two stems, and the leaves of one were lightly rubbed with bleached, nonabsorbent cotton. If only one stem sprouted, half the leaves were rubbed. Shortly after rubbing, the plants were sprayed: four with a solution of PS-85B (2.2×10^4 conidia per milliliter), four with *S. botryosum* EGS 04-118 (2.9×10^4 conidia per milliliter), and four with *S. vesicarium* EGS 04-001 (2.8×10^4 conidia per milliliter). Four plants also were sprayed with the control solution. All plants were sprayed to runoff.

The sprayed plants were placed in a dew chamber at 20 C with a 14-hr photoperiod of fluorescent light for 72 hr to ensure favorable conditions for infection. The plants then were placed in a greenhouse until symptoms were visible (4–5 days), and each leaf was scored for the presence or absence of chlorotic oval lesions. No attempt was made to rate severity of infection because it was felt that the difficulty of getting uniform wetting of each leaf would make any severity rating somewhat inaccurate. Some symptomatic leaves from each plant were subsequently placed in moist chambers (100-mm petri dishes containing filter paper disks moistened with distilled water) for 5 days to induce sporulation; then spores were examined and measured.

Relative virulence of onion and asparagus isolates. A similar experiment was done to compare the reactions of onion plants inoculated with PS-85B and the isolate of *S. vesicarium* from asparagus. Some leaves of each plant were rubbed as before. Four plants were sprayed with PS-85B (6.3×10^4 conidia per milliliter), four with Sv(asp) (8.8×10^4 conidia per milliliter), and four with the control solution. They were placed in the dew chamber and evaluated as in the previous experiment, except that all leaves were placed in moist chambers.

Another experiment compared the reaction of onion plants inoculated with PS-85B and the isolate of *S. vesicarium* from Texas, Sv(tex). Some leaves of each plant were rubbed as before. Four plants were sprayed with PS-85B (2.8×10^4 conidia per milliliter), four with Sv(tex) (4.0×10^4 conidia per milliliter), and four with the control solution. They were placed in the dew

chamber and scored as in the previous experiment.

Effect of plant and leaf age on susceptibility to Stemphylium leaf blight. An experiment was done to determine the reaction on onion plants of different ages after inoculation with conidia of PS-85B. Three mature onion plants with flower stalks, three sprouted bulbs with 4- to 8-wk-old leaves, and 30 seedlings in the three-leaf stage (9–12 wk old) were inoculated with conidia of PS-85B at a concentration of 1.5×10^4 conidia per milliliter. Before inoculation, some of the leaves of plants with flower stalks and those on resprouted bulbs were rubbed with bleached, nonabsorbent cotton as described previously. Half of the seedlings were rubbed on the second leaf. Two plants with flower stalks, three from sprouted bulbs, and 30 seedlings were treated similarly except that they were sprayed with the control solution. All plants were placed in a dew chamber. This experiment was repeated with two plants with flower stalks, six from sprouted bulbs, and 60 seedlings with rubbed and nonrubbed leaves. Half of the plants were inoculated with PS-85B at a concentration of approximately 1.3×10^4 conidia per milliliter and the other half were sprayed with the control solution as before.

To compare the susceptibility of the two youngest and two oldest leaves per stalk, four bulbs that had been sprouted 4 wk earlier were inoculated with a solution of 3.8×10^4 conidia per milliliter of PS-85B and four bulbs with the control solution. Some leaves of all plants were rubbed with bleached, nonabsorbent cotton before inoculation. Plants were placed in the dew chamber for 72 hr. Leaves were scored for symptoms after 1 wk in the greenhouse. This experiment was replicated once.

Effect of duration of leaf wetness on disease severity. A time course experiment was run to determine the duration of leaf wetness necessary after inoculation for disease development. Two leaves of the same age were selected from each of 40 bulbs with 2- to 3-wk-old foliage; one leaf was rubbed with bleached, nonabsorbent cotton. The rubbed and nonrubbed leaves of 20 plants were inoculated with PS-85B (9.6×10^3 conidia per milliliter). Twenty other plants were treated similarly, except they were sprayed with the control solution. All 40 plants were randomly arranged in a mist chamber at 20 C with a photoperiod of 12 hr of fluorescent light. Five plants from each treatment, inoculated and control (a total of 10 plants), were removed after 3, 6, 24, and 36 hr and placed in the greenhouse. After 4 days in the greenhouse, lesions were counted on the rubbed and nonrubbed leaves, and the number of lesions per centimeter of leaf length (les/cm) was counted and plotted against time. The slope, les/cm/hr, for rubbed and nonrubbed leaves was compared by using the T-test.

An additional time course experiment was done by spraying PS-85B at a higher spore concentration (8.4×10^4 conidia per milliliter) on 16 onion plants and spraying 20 others with the control solution. Plants were kept in the mist chamber for 6, 12, 18, or 24 hr. Four inoculated plants were removed at 6 hr, five at 12 hr, four at 18 hr, and three at 24 hr after inoculation. Five control plants were removed at each time interval.

RESULTS

Taxonomy. Simmons (9) described the conidia of *S. vesicarium* as being oblong to oval with 1–5 transverse septa, sometimes constricted at the middle one or three most central of these, and with a complete or nearly complete series of longitudinal septa. The conidia range in size from 25–42 μm × 12–22 μm (average = 33.4 × 17.7 μm) with a l:w ratio of 1.5–2.7 (average = 1.9) from host tissue and 2.5–3.0 in culture. *S. botryosum* has subspherical to oblong spores strongly constricted at the middle septum, 33–35 μm × 24–26 μm with a l:w ratio of 1.0–1.5 from host tissue, 1.5 in culture (9).

The conidia of PS-85B most closely resembled *S. vesicarium*, although individual conidia varied in size and number of constricted transverse septa. Conidia were 34–38 μm × 15–22 μm (average = 35.6 × 16.8 μm) with a l:w ratio of 2.1–2.4 for conidia from host tissue and 2.0–2.3 from culture (Table 1). Dimensions of the other *Stemphylium* isolates studied are also presented in Table 1. As is evident, the dimensions of spores vary widely, but the l:w ratio is fairly consistent within each species and is a good method of identification.

Simmons (9,10) described the perfect state of *S. botryosum* as maturing slowly in culture, forming thick-walled pseudothecia in 2–12 mo (8). Ascospores are brown, muriform, 40 × 17 μm, and blunt on top and flat on the bottom. Typically, pseudothecia of the isolates of *S. vesicarium* utilized matured after 3–6 mo. The ascospores were 38 × 18 μm, often pointed at the top. The perfect state of Prattsburg isolates developed readily on onion leaf agar in 2–3 mo. The ascospore were 30.6 ± 2.0 μm × 11.6 ± 1.0 μm and often pointed.

When 50 conidia of *S. botryosum* were compared to 53 conidia of *S. vesicarium* and 52 conidia of PS-85B grown on onion leaf agar under the same conditions, the conidia of *S. botryosum* measured 30 ± 3 μm × 18 ± 2 μm, those of *S. vesicarium* 34 ± 5 μm × 17 ± 3 μm, and those of PS-85B 36 ± 5 μm × 17 ± 2 μm. The l:w ratio of the conidia of *S. botryosum* proved to be significantly different from those of *S. vesicarium* and PS-85B, whereas l:w ratios of the latter two did not differ significantly from each other ($P < 0.05$) (Table 2).

Relative virulence of onion and asparagus isolates. When onion plants inoculated with isolates of *S. vesicarium*, *S. botryosum*, and PS-85B were incubated in a dew chamber for 72 hr and then allowed to develop symptoms in the greenhouse, the leaves of the plants inoculated with *S. vesicarium* and PS-85B showed flecking and oval lesions. The leaves of the plants inoculated with *S.*

TABLE 1. Conidial dimensions with standard deviations and length-to-width ratios (l:w) of isolates compared with those dimensions found for each species by Simmons (9)[a]

Isolate	Average conidial dimensions in culture[b] length × width (μm)	l:w	Average conidial dimensions on host[b] length × width (μm)	l:w
S. vesicarium Simmons (9)	45 × 18	2.5–3.0	33.4 × 17.7	1.9
S. botryosum Simmons (9)	33 × 23	1.5	34 × 25	1.0–1.5
EGS 24-001 (*S. vesicarium*)	33 ± 4 × 14 ± 2 (n = 33)	2.4 ± 0.4	32 ± 4 × 12 ± 2 (n = 60)	2.7 ± 0.5
EGS 04-118 (*S. botryosum*)	30 ± 4 × 18 ± 3 (n = 44)	1.7 ± 0.3	23 ± 3 × 15 ± 2 (n = 35)	1.5 ± 0.2
PS-85B	35 ± 6 × 17 ± 2 (n = 50)	2.3 ± 0.5	36 ± 4 × 15 ± 2 (n = 70)	2.4 ± 0.5
Sv(asp) (*S. vesicarium*)	36 ± 6 × 17 ± 2 (n = 55)	2.1 ± 0.4	32 ± 5 × 16 ± 2 (n = 51)	2.1 ± 0.3
Sv(tex) (*S. vesicarium*)	34 ± 2 × 17 ± 2 (n = 51)	2.0 ± 0.4	25 ± 4 × 14 ± 2 (n = 51)	1.8 ± 0.3

[a] Conidia were produced either from colonies grown on potato-dextrose agar or from diseased onion leaves grown in moist chambers.
[b] n = number of spores measured.

botryosum had a few lesions. Control plants had no lesions. Leaves from the plants inoculated with *S. vesicarium* or PS-85B that were placed in moist chambers yielded conidia characteristic of *S. vesicarium*. Lesions from the *S. botryosum*-inoculated plants yielded conidia characteristic of *S. botryosum*.

Onion plants inoculated with conidia of Sv(asp) or conidia of PS-85B showed small chlorotic flecks or larger water-soaked lesions. With PS-85B, conidia soon covered the symptomatic leaves when they were placed in moist chambers, and the perfect state also was observed to develop in the leaf tissue. In plants inoculated with Sv(asp), conidia were produced on leaves in a moist chamber, but no sign of the perfect state was observed. The control plants developed no symptoms of disease.

The Texas isolate caused the same symptoms on onion as PS-85B. In moist chambers, conidia were produced on symptomatic leaves, and the perfect state was present for both isolates. The control plants developed no symptoms of disease.

Effect of plant and leaf age on susceptibility to Stemphylium leaf blight. All ages of onion plants were affected by inoculation with PS-85B (Table 3). Rubbed leaves developed more lesions than nonwounded leaves. The control plants did not show any symptoms of disease.

When onion plants of the same age were inoculated, there was a slightly greater incidence of disease on rubbed leaves compared with nonrubbed leaves, and a greater incidence of disease on the two oldest leaves compared with the two youngest leaves (Table 4).

Effect of duration of leaf wetness on disease severity. The longer plants were left in a mist chamber after inoculation with the pathogen, the greater the number of lesions per centimeter of leaf. Rubbed leaves had significantly more lesions per centimeter than nonrubbed leaves (Fig. 1). This was true at both concentrations of inoculum sprayed on plants. The results indicate that disease can occur after 18–24 hr of exposure to moisture after inoculation.

DISCUSSION

The *Stemphylium* sp. isolated from lesions on leaves of commercially grown onion plants in New York fits the description of *S. vesicarium*. The fungus caused similar symptoms when reinoculated on onion plants in the greenhouse. It caused disease on plants of all ages, and older leaves were more frequently infected.

Falloon et al (2) inoculated nonwounded asparagus plants with an isolate of *S. vesicarium* from onion and no lesions developed. We inoculated both wounded and nonwounded onion plants with an isolate of *S. vesicarium* from asparagus, and similar symptoms resulted as when the isolate from onion was used. Clearly, more experimentation is needed to clarify the specificity of this fungus.

Disease severity was greater when onion leaves were either rubbed with bleached, nonabsorbent cotton or exposed to longer periods of free moisture after inoculation with conidia of *S. vesicarium*. These results agree with time course experiments with *S. vesicarium* on asparagus, in which more lesions formed on wounded plants than on nonwounded plants, and more lesions formed after 24 hr of stem wetness than at 3, 4, 6, or 12 hr (3). Whereas abrasion of the leaf surface increased disease severity, the pathogen did not need a wound or rupture of the cuticle to penetrate. It has been shown on several previous occasions that penetration is almost exclusively through stomata (1,2).

Miller et al reported in 1976 that losses in Texas onion crops due to *S. vesicarium* were as high as 90%, with most damage occurring after rains lasting more than 24 hr (6). From this, and from our observations, one can conclude that the warm, humid summers of New York would be conducive to the serious epidemics of Stemphylium leaf blight caused by *S. vesicarium*.

TABLE 2. Analysis of variance of the length-to-width (l:w) ratio of conidia of three isolates of *Stemphylium* by using the Student-Newman-Keuls test ($P < 0.05$)

Isolate	Number of spores	Mean l:w ratio[a]	Standard deviation
S. botryosum			
EGS 04-118	50	1.68 b	0.19
S. vesicarium			
EGS 24-001	53	2.02 a	0.41
PS-85B	52	2.08 a	0.35

[a] Means followed by the same letter are not significantly different at $P = 0.05$.

TABLE 3. Proportion of rubbed and nonrubbed onion leaves from plants of different ages showing symptoms after being sprayed with approximately 1×10^4 conidia per milliliter of *S. vesicarium* (INOC) or sprayed with a solution of distilled water and Tween 20 alone (CONT)[a]

	Flowering		Vegetative		Seedling	
Treatment	Infected lvs/total	Percentage	Infected lvs/total	Percentage	Infected lvs/total	Percentage
INOC						
Rubbed	11/18.5	59	7.5/15.5	48	8/14.5	55
Nonrubbed	3.5/20	18	2/18	11	4.5/18	25
CONT						
Rubbed	0/10.5	0	0.5/13[b]	3[b]	0/14.5	0
Nonrubbed	0/12	0	0/15	0	0/14.5	0

[a] Two replicates.

[b] One leaf of a control plant had a few lesions from which *S. vesicarium* was isolated after incubation in a moist chamber. Presumably, the plant brushed against an inoculated plant in the dew chamber where the plants were randomly arranged.

TABLE 4. Proportion of symptomatic leaves (out of total leaves) of onion plants sprayed with 3.8×10^4 conidia per milliliter of *S. vesicarium* or sprayed with a solution of distilled water and Tween 20 for rubbed and nonrubbed leaves and for oldest and youngest leaves[a]

	Inoculated		Control	
	Symptomatic/ total leaves	%	Symptomatic/ total leaves	%
Rubbed leaves	18.5/26.5	70	0/22	0
Nonrubbed leaves	15.5/26.0	60	0/24	0
Oldest two leaves (rubbed and nonrubbed)	19.5/21.5	91	0/15	0
Youngest two leaves (rubbed and nonrubbed)	5.5/21.0	26	0/15	0

[a] Two replicates.

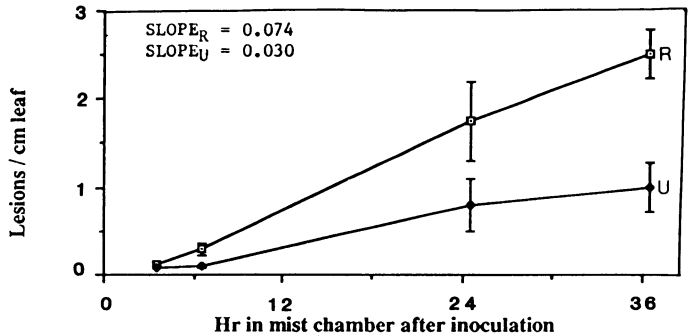

Fig. 1. The effect of duration of leaf wetness after inoculation on the number of lesions per centimeter of leaf length shown for rubbed and nonrubbed onion leaves sprayed with 9.6×10^3 conidia per milliliter of PS-85B. Control plants are not shown. R = leaves rubbed with cotton wool; U = leaves nonrubbed. Bars represent ± standard error. Slopes were significantly different at $P < 0.02$.

LITERATURE CITED

1. Cowling, W. A. 1980. Environmental, genetic, and physiological factors influencing disease severity in Stemphylium leafspot of alfalfa in California. Ph.D. thesis, University of California, Davis. 211 pp.
2. Falloon, P. G., Falloon, L. M., and Grogan, R. G. 1987. Etiology and epidemiology of Stemphylium leaf spot and purple spot of asparagus in

California. Phytopathology 77:407-413.
3. Johnson, D. A., and Lunden, J. D. 1986. Effects of wounding and wetting duration on infection of asparagus by *Stemphylium vesicarium*. Plant Dis. 70:419-420.
4. Johnson, D. A., and Lunden, J. D. 1984. First report of purple spot (*Stemphylium vesicarium*) of asparagus in Washington. Plant Dis. 68:1099.
5. Lacy, M. L. 1982. Purple spot: A new disease of young asparagus spears caused by *Stemphylium vesicarium*. Plant Dis. 66:1198-1200.
6. Miller, M. E., Taber, R. A., and Amador, J. A. 1978. Stemphylium blight of onion in south Texas. Plant Dis. 62:851-853.
7. Rao, N. N. R., and Pavgi, M. S. 1975. Stemphylium leaf blight of onion. Mycopathologia 56:113-118.
8. Sherf, A. F., and MacNab, A. A. 1986. Vegetable diseases and their control. 2nd ed. John Wiley and Sons, New York. 728 pp.
9. Simmons, E. G. 1969. Perfect states of *Stemphylium*. Mycologia 61:1-26.
10. Simmons, E. G. 1985. Perfect states of *Stemphylium*. II. Sydowia 38:284-293.
11. Teodora, N. G. 1922. Pathogenicity of *Macrosporium parasiticium*. Phytopathology 12:20.