Effects of Wounding and Wetting Duration on Infection of Asparagus by *Stemphylium vesicarium*

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ABSTRACT

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Wounding was not required for infection on asparagus by *Stemphylium vesicarium*. However, infections were more numerous and occurred at shorter wetting durations on wounded than on nonwounded plants. Wounds produced by blowing sand were entry points for infection when infection occurred 24–48 hr after injury.

Purple spot, caused by *Stemphylium vesicarium* (Wallr.) Simons, was recently found to damage marketable spears of asparagus (*Asparagus officinalis* L.) before harvest in Washington State (4), Michigan (5), California (3), and New Zealand (2). Economic loss is due to small (1–2 mm), sunken, purple lesions on spears that are usually most prevalent after cool, wet spring weather. Spears with lesions are rejected from fresh-market sales. *S. vesicarium* also infects the asparagus fern after harvest. In Michigan, infection was reported to occur only through wounds on excised spears (1,5). In Washington, more lesions occurred on potted asparagus wounded by sand blasting than on plants not wounded before inoculation (4). This study was conducted to compare infection by *S. vesicarium* on wounded and nonwounded asparagus at various durations of wet periods and at various times of wounding before inoculation.

MATERIALS AND METHODS

Plants of the asparagus cultivar Mary Washington were grown from seed in 11.4-cm-diameter pots in a silt loam-sand-peat (1:1:1) mix in the greenhouse. Plants were fertilized with a 16-16-16 (NPK) fertilizer at 4-wk intervals. Natural light supplemented with fluorescent lamps was used to provide a photoperiod of 16 hr/day.

Shoots were cut at soil level and allowed to regrow before each inoculation. Shoots were 2–7 wk old (mean length 29–48 cm) and crowns were 3–6 mo old when inoculated. Crowns and shoots within each of four inoculation tests were the same age.

Plants were wounded by making a single vertical pass 45 cm from each plant with a hand-held sand blaster (Seedaire Model 22632A, Dayton Electric Manufacturing Co., Chicago, IL), using 16-grit silica sand. Air pressure of the sand blaster was set at 124 kPa, creating a wind velocity of about 14 km/hr at plant level. Plants were tied to bamboo stakes for stability during wounding. Each pass took about 0.4 sec to complete.

Conidia for inoculation were produced on Difco potato-dextrose agar medium in petri dishes placed under continuous fluorescent light at 20–23 C for 10–14 days. Conidia were scraped and washed from the agar, filtered through cheesecloth, and sprayed on test plants ($2.2–4.2 \times 10^4$ conidia per milliliter) with a 4-L hand-pump garden sprayer (Universal-Gerwin, Saranac, MI) until inoculum dripped from plants. One drop of Tween 20 per 500 ml of water was added to the inoculum.

After inoculation, plants were placed in a plastic mist chamber for 3–24 hr, removed, and dried as quickly as possible with forced air from a fan, then placed in the greenhouse. The mist chamber was $1.8 \times 0.9 \times 1.2$ m (high), and a fine mist was supplied for 1 min every 10 min. Fluorescent and incandescent lamps next to the mist chamber gave a light intensity inside the chamber of about 24 μmol photon m^{-2} s^{-1}.

The length of each inoculated shoot was measured, and 12–15 days after inoculation, lesions were counted only on the primary axis of each shoot. The number of lesions per linear centimeter of stem was tabulated for each shoot, and a mean was calculated for each pot. Experiments were set up in factorial arrangements. Data were analyzed by analysis of variance and linear regression. Four experiments were carried out. Concentrations of conidia in the inoculum were not standardized between experiments, so comparisons were made only within an experiment.

Length of wet period. To determine the effect of length of wet period on infection by *S. vesicarium*, plants were wounded or not wounded, inoculated with a conidial suspension (36,000 conidia per milliliter), and removed from the mist chamber 3, 4, 6, 12, and 24 hr after inoculation. The time that elapsed from washing conidia from agar to inoculation was 35 min. Tween 20 was not added to the inoculum. Three replicates with two plants per replicate and two stems per plant were used. Temperature during the mist period was 15 C. Mean temperatures in the greenhouse were 21 C during the day and 18 C at night.

To determine the effect of light and length of wet period on infection, half of the mist chamber was covered with black plastic to exclude light. Plants were wounded or not wounded, inoculated with 42,000 conidia per milliliter at 1030 hours, and plants were removed from the dark and lighted sections of the mist chamber after 3, 4, 6, 12, and 24 hr. The time that elapsed from washing the conidia from agar to inoculation was 75 min. Five replicates were used with two plants per replicate and three stems per plant. Temperature during the mist period was 20 C. Mean temperatures were 21 C during the day and 18 C at night.

Time of wounding. To determine the effect of time of inoculation after wounding, plants were wounded or not

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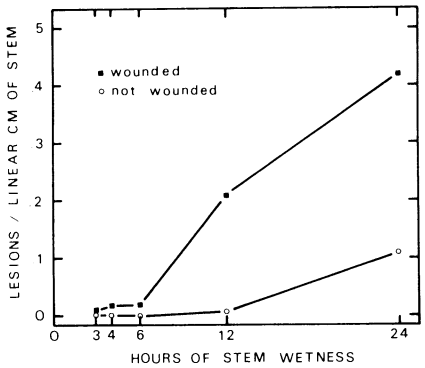

Fig. 1. Lesions per linear centimeter of asparagus stems not wounded and wounded with air-driven sand, inoculated with *Stemphylium vesicarium*, and placed in a mist chamber for 3–24 hr.

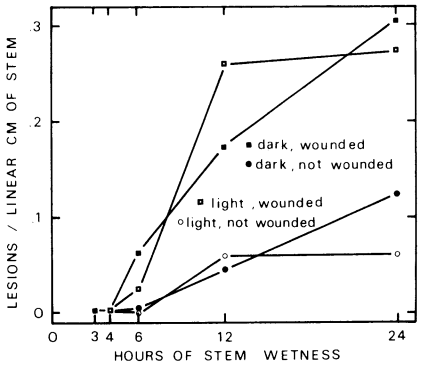

Fig. 2. Lesions per linear centimeter of asparagus stems not wounded and wounded with air-driven sand, inoculated with *Stemphylium vesicarium*, and placed in a mist chamber in light and darkness for 3–24 hr.

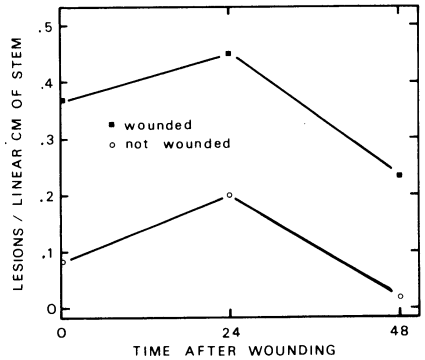

Fig. 3. Lesions per linear centimeter of asparagus stems not wounded and wounded with air-driven sand and inoculated with *Stemphylium vesicarium* 0, 24, and 48 hr after wounding.

wounded, then inoculated 0, 24, and 48 hr after wounding. Inoculum was adjusted to 28,000 conidia per milliliter for each of the three inoculations. Plants were in the mist chamber for 24 hr. Temperature was 26 C during the wet period. Mean temperatures in the greenhouse were 22 C during the day and 16 C at night.

Plants were also wounded 96, 72, 48, 24, and 0 hr before inoculation. Nonwounded and wounded plants were inoculated at the same time with 22,000 conidia per milliliter and placed in the mist chamber for 24 hr. A randomized complete block design with six replicates was used. Temperature during the mist period was 23 C. Mean temperatures in the greenhouse were 23 C during the day and 15 C at night.

RESULTS

Wounded and nonwounded plants became infected in all experiments (Figs. 1–3); however, in each experiment, wounded plants had significantly more lesions ($P = 0.01$) than nonwounded plants. The number of lesions (dependent variable) significantly increased ($P = 0.01$) in both the wounded and nonwounded treatments as the wet period (independent variable) increased from 3 to 24 hr (Figs. 1 and 2). The shortest wet period during which lesions appeared on wounded plants was 3 hr after both inoculations to determine the effect of length of wet period on infection, and on nonwounded plants, it was 12 hr after the first inoculation and 4 hr after the second. There was not a significant difference ($P = 0.05$) in numbers of lesions between the light and dark treatments (Fig. 2), as shown by the F value in the analysis of variance.

Time of wounding to inoculation from 0 to 48 hr had no effect on disease development (Fig. 3). Linear regressions for time of inoculation after wounding (independent variable) on lesions per linear centimeter of stem (dependent variable) were not significant ($P > 0.05$) for both the wounded and nonwounded treatments. When tested with single degree-of-freedom contrasts, the wounded and nonwounded treatments differed significantly ($P = 0.01$) at each of the three dates of inoculation after wounding.

Plants wounded 96, 72, 48, 24, and 0 hr before inoculation had 0.09, 0.07, 0.04, 0.13, and 0.22 mean lesions per linear centimeter of stem, respectively. The nonwounded check had a mean of 0.03 lesions per linear centimeter. Plants wounded 0 and 24 hr before inoculation had significantly more ($P = 0.01$) lesions per linear centimeter of stem than the nonwounded plants when tested with single degree-of-freedom contrasts. Numbers of lesions on plants wounded 48, 72, and 96 hr before inoculation did not differ significantly from those on nonwounded plants.

DISCUSSION

S. vesicarium infected potted asparagus without prior wounding. This is in contrast to wounds being required for infection on excised spears (1,5). Infections were more numerous and occurred at shorter wetting durations on wounded than on nonwounded plants, because wounding creates additional avenues for infection. The probability of a relatively early infection would probably increase with an increase in the number of infection sites. Wounds remained entry points when injury occurred up to 24–48 hr before inoculation.

Wounding asparagus by air-driven sand as we did ensured breaks in the epidermis for infection. Pinpoint wounds were formed, and sometimes portions of the epidermis were removed. This wounding was more severe than we normally observe in the field. Wounding by blowing sand could have the potential to increase disease severity, especially during relatively short wet periods and when asparagus is grown in sandy soils, similar to that found in parts of the Columbia Basin in central Washington. However, we have not established the importance of wounding by blowing sand on the development of purple spot in the field in Washington. As demonstrated, wounds are not needed for disease development.

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