Etiology and Epidemiology of Stemphylium Leaf Spot and Purple Spot of Asparagus in California

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ABSTRACT

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Stemphylium vesicarium was isolated from all asparagus production areas throughout California except the Imperial and Coachella valleys. Most California isolates from spears, green fern, or debris from the previous summer's growth as well as three isolates of *S. vesicarium* from Switzerland and two isolates resembling the conidial state of *S. majusculum* from asparagus in England were pathogenic on young asparagus seedlings. Isolates of *Alternaria* spp. from asparagus in California and England and one isolate of *S. vesicarium* from onions did not cause disease on asparagus seedlings. Disease ratings of spears harvested from the field during spring of 1983 and 1984 indicated that disease was more severe following periods of wet weather or sprinkler irrigation. Symptoms on spears were reproduced without prior wounding of spears, and disease was more severe when high humidity conditions were extended from 24 to 48 or 72 hr after inoculation. This is contrary to earlier reports suggesting that sand-blasting is requisite for disease development. All penetrations of tissue from germinated spores of *Stemphylium* and *Pleospora* were exclusively through stomata. Stomata started to close within 5 min after harvest of spears and all were closed within 60 min. Thus, reproduction of spear symptoms failed unless water stress was relieved or prevented before inoculation. Pseudothecia of *Pleospora* on fern debris were shown to be an important source of inoculum for purple spot of asparagus spears.

The first reported association of *Stemphylium* with asparagus (*Asparagus officinalis* L.) was in 1833 when Wallroth named the genus *Stemphylium* (22). The specimen from which Wallroth described the type species, *Stemphylium botryosum* Wallr. (*Pleospora herbarum* (Pers. ex Fr. Rabenh.)), consisted of four pieces of asparagus stem. Thus, asparagus is the "type host."

Stemphylium was first described as a pathogen of asparagus in Japan in 1973 when *S. botryosum* was reported as the cause of Stemphylium leaf spot (17,21). Elsewhere *S. vesicarium* (Wallr.) Simmons (19) has been shown to cause both a foliar and spear disease (purple spot) of asparagus, e.g., in California (7), Washington (9,10), Michigan (5,11), England (D. Ellerton, *personal commmunication*), and New Zealand (6,15,20), and Stemphylium leaf spot has been reported in France (2) and Switzerland (8).

Purple spot was chosen as a common name (11) because spear infections result in numerous, slightly sunken, purplish spots often occurring on only one side of the spear (7,11). Reports from Michigan (5,11) indicate that symptoms on excised spears could be reproduced only when conidial inoculation was preceded by wounding the surface of the spears. Therefore, it was suggested (11) that purple spot was associated with sand-blasting of the spears and that the physical damage from impacting sand and soil was a prerequisite for disease development.

This paper reports the field conditions under which purple spot occurs on asparagus in California, the conditions necessary for reproduction of symptoms on spears without wounding, and the distribution, relative virulence, and identity of isolates of the pathogen collected from asparagus production areas of California and other parts of the world.

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MATERIALS AND METHODS

Isolation, identification, and distribution of the pathogen. *Stemphylium* was first isolated from asparagus spears in California after periods of wet weather during the harvest season in 1982 (7). In 1983 and 1984, samples of fern debris from the previous year's fern growth, diseased green fern, or diseased spears were collected from 33 asparagus fields throughout California. The pathogen was isolated as described by Lacy (11). Diseased spears, green fern, or debris were surface sterilized in 0.5% sodium hypochlorite for 15 min, then purple lesions on spears or green fern were excised and plated on potato-dextrose agar (PDA). The pseudothecia of *Pleospora* on the surface of debris (5,7) were scraped off with a sterile scalpel and plated on PDA. Plates were incubated in a 20 C incubator with an 18-hr photoperiod under fluorescent lights.

For identification of the pathogen, pure cultures were transferred to 86-mm-diameter petri dishes containing 20 ml of 5% V-8 juice agar (50 ml of V-8 juice and 20 g of Difco bacto-agar in 950 ml of distilled water) adjusted to pH 7.5 with AR-grade calcium carbonate powder and incubated in a 20 C incubator with an 8-hr photoperiod (20). The technique of Luttrell (13) was used to induce synchronous sporulation that enabled preparation of slides with spores at a similar stage of development. The method involved cutting 1.5-cm^2 plugs from the periphery of 5-day-old colonies growing on 5% V-8 juice agar. The plugs were transferred to the surface of water agar plates that were kept at 20 C under continuous light for approximately 24 hr (20). Slides of conidia and conidiophores were prepared by mounting thin slices of the sporulating culture in lactophenol.

Pathogenicity of all *Stemphylium* as well as several *Alternaria* isolates obtained from spears or debris was determined on 8.5-wk-old asparagus seedlings. Six seeds of the cultivar U.C. 157 were sown in U.C. mix (14) in each of a number of 100-mm-diameter pots. After 6 wk of growth, the shoots were trimmed to soil level and allowed to regrow. Inoculum was prepared from cultures grown in 86-mm-diameter petri plates containing 20 ml of 20% V-8 juice agar (200 ml of V-8 juice mixed with 3 g of calcium carbonate, 20 g of Difco bacto-agar and 800 ml of distilled water). The plates

were incubated for 14 days in a 25 C incubator with a 16-hr photoperiod under fluorescent lights. Approximately 10 ml of sterile distilled water and one drop of Tween-20 (polyoxyethylene sorbitan mono-oleate) was added to each plate and the conidia were dislodged by gently rubbing a sterile L-shaped glass rod over the surface of the plate. The resulting conidial suspension was filtered through four layers of cheesecloth and adjusted to 10^5 conidia per milliliter. Compressed CO_2 was used to spray the inoculum through an atomizer onto the seedlings until runoff. Control seedlings were sprayed with a mixture of sterile distilled water and Tween-20. Immediately after inoculation each seedling pot was covered with a plastic bag for 48 hr to maintain high relative humidity. Inoculation and incubation were carried out in a growth cabinet in the middle of a 24-hr light period (Fig. 1). The light/dark temperature of the growth cabinet varied from 15.5 C to 13.5 C, respectively, and light intensity at plant height was approximately 150 microeinsteins, $m^{-2}s^{-1}$, which is equivalent to approximately 63% of the outdoor light intensity on a cloudy day in Davis. These conditions were maintained in all growth cabinet experiments unless otherwise stated. The seedling pots were arranged in the growth cabinet in a randomized complete block design with three replicates of each isolate.

Eight days after inoculation the seedlings were evaluated using the following visual rating scale: 1 = no lesions; 2 = lesions on main stems only; 3 = lesions on main stems and secondary stems; 4 = lesions on main stems and secondary stems and slight cladophyll drop; and 5 = lesions on main stems, secondary stems, and severe defoliation.

Data were subjected to analysis of variance and differences between means were determined using an unrestricted least significant difference test.

Field observations. In 1983 spears were harvested from a plot of the cultivar U.C. 157, in which fern debris had been left on the soil surface. The plot consisted of 16–28.6-m-long rows growing 1.5 m apart. The soil type was Yolo loam (sand 30–50%, silt 30–50%, clay 10–25%). Spears were harvested three times each week starting on 6 March and ending on 18 May. After each harvest, between 11 and 867 spears were trimmed to 225 mm and evaluated for disease using two methods: the length from the tip of each spear to the first lesion was measured, and a visual rating, based on the number of lesions on each spear was used to grade the spears into the following disease classes: 0 = no symptoms; 1 = 1–20 lesions per spear, few with purple margins; 2 = 21–50 lesions per spear, all with purple margins; 3 = 51–90 lesions per spear, all with purple margins; 4 = more than 90 lesions per spear, unmarketable.

A disease severity index (DSI) ranging from 0 (no disease on any spears) to 100 (all spears in disease class 4), was calculated for each harvest using the formula of Sherwood and Hagedorn (18):

$$\text{DSI} = \frac{\Sigma\ (\text{disease class} \times \text{no. of spears in that class})\ 100}{\text{total no. of spears} \times 4}$$

In 1984, two plots of U.C. 157 were established approximately 300 m apart. Supplemental water was applied at the rate of approximately 2.5 mm/hr through sprinkler irrigation lines to one plot on 6 March (25 mm), 9 March (25 mm), 28 March (9 mm), and 8 April (25 mm). Spears were harvested three times per week and evaluated as in 1983. Rainfall, evaporation, and maximum and minimum air temperature data were collected from the Davis Climatic Benchmark Station approximately 0.5 km from the trial site.

Rainfall and evaporation data and the difference between rainfall and evaporation were correlated with DSI and the mean length from the tip to the first lesion.

Reproduction of symptoms in vitro and in vivo. Disease-free spears of U.C. 157 were cut in the field and either a) placed in a clear plastic container on paper towels moistened with distilled water or b) placed immediately in water. Five spears were then transferred to 850-ml plastic containers (100 mm square × 103 mm deep) that had been filled with distilled water. The lower 25–50 mm of each spear was cut off under water and quartz sand was gently poured around the base of the spears in each container to provide support for the spears.

Spears were inoculated with a conidial suspension with 10^5 *Stemphylium* conidia per milliliter. Inoculations were done under the same conditions as the pathogenicity test on seedlings. The clear plastic containers were covered with clear plastic and each 850-ml container was placed in a plastic bag and returned to the growth cabinet; bags were removed after 24, 48, or 72 hr. The same method of inoculation was used to reproduce symptoms on spears of plants growing in pots of U.C. mix.

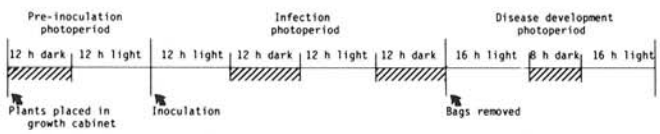

Fig. 1. Outline of the light/dark sequence before inoculation and during infection and disease development.

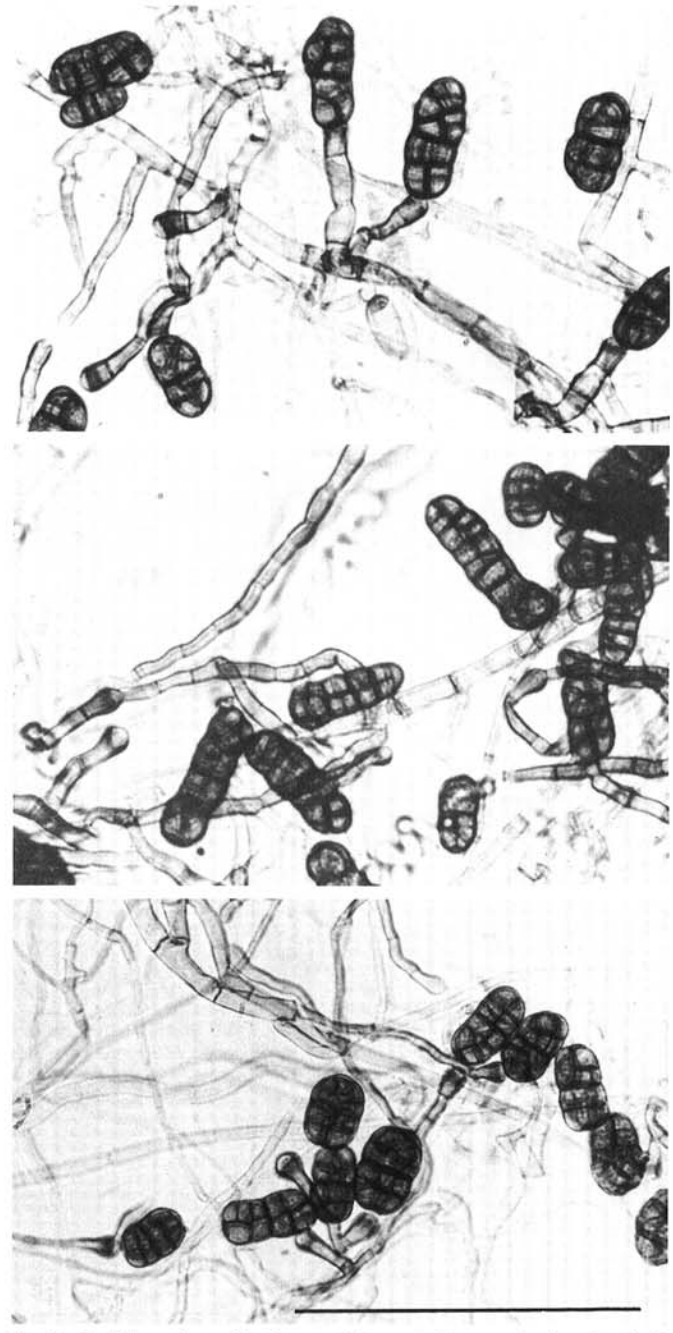

Fig. 2. Conidia and conidophores of *Stemphylium vesicarium* (top). *S. majusculum* (middle), and *S. botryosum* (bottom) isolated from asparagus in California and England, respectively. Scale bar = 100 μm.

To determine if fern debris was the source of field inoculum, five or six pieces of debris bearing mature pseudothecia of *Pleospora* were inserted vertically around excised spears in saturated sand or spears from plants growing in pots of U.C. mix. The spears and debris were covered with plastic bags for 24, 53, 75, or 98 hr, during which time the inside of the bags were regularly misted every 2 to 4 hr with sterile distilled water applied through an atomizer.

Scanning electron microscopy (SEM). To determine the means by which germ tubes from *Stemphylium* conidia or ascospores of *Pleospora* penetrate asparagus spears, individual lesions were excised from diseased spears collected either from the field or from growth cabinet experiments. The tissue was fixed in FAA (10 ml of Formalin, 10 ml of glacial acetic acid, 90 ml of ethanol, and 90 ml of distilled water), thoroughly rinsed with distilled water, and prepared for SEM by dehydration in acidified 2,2-dimethoxypropane (12) followed by critical-point drying with CO_2. Tissue prepared in this manner was mounted on studs, coated with 40–60 nm of gold in a sputter coater, and examined with an I.S.I. D.S.-130 SEM operating at 10 or 14 kV.

Time for stomatal closure after harvest. An initial study indicated that stomata on asparagus spears closed soon after harvest. To determine how long stomata remained open, approximately 100 spears were obtained from the field. Epidermal strips were taken by coating microscope slides with Duro Super Glue and pressing each slide against the surface of the spear for approximately 10 sec. Epidermal strips that adhered to the slides were viewed under a light microscope and the proportion of open stomata was determined by observing 100 stomata from strips taken before the spears were harvested and at 5, 10, 15, 30, 60, 120, and 240 min after harvest.

RESULTS AND DISCUSSION

Stemphylium sp. was isolated from an average of 76% of purple lesions on asparagus spears, 91% of lesions on green fern and 65% of scrapings from fern debris, whereas *Alternaria* spp. were isolated from about 12%, 9%, and 33% of purple spots, fern lesions, or debris scrapings, respectively. All 24 *Stemphylium* isolates from California and three isolates from Switzerland were identified as *S. vesicarium* (19). On the basis of spore size, shape, and septation, two isolates from England were similar to *S. majusculum* described by Simmons (19) (Fig. 2, Table 1). Conidia of *S. botryosum* were different from those of *S. vesicarium* (Fig. 2) and could be separated on the basis of smaller length/width ratio (Table 1). Most isolates of *S. vesicarium* and both isolates of *S. majusculum* from asparagus were pathogenic on asparagus seedlings. Mean disease scores ranged from 1.00 to 4.33 for *S. vesicarium* and from 3.00 to 3.67 for *S. majusculum*. One culture of *S. vesicarium* isolated from onions in California and eight isolates of *Alternaria* spp. did not cause disease on asparagus seedlings. *S. vesicarium* has been reported as a pathogen of asparagus in California (7), Michigan (5,11), Washington (9,10), New Zealand (6,15,20), and France (2), but *S. botryosum* was reported as causing Stemphylium leaf spot on asparagus in Switzerland (8) and Japan (17,21). The dimension, length/width ratio, number of lateral constrictions, and number of longitudinal and transverse septa of

Fig. 3. Distribution of *Stemphylium vesicarium* in asparagus fields in California during 1983 and 1984. • = *S. vesicarium* isolated and □ = *S. vesicarium* not isolated.

TABLE 1. Conidial characteristics of 32 isolates of *Stemphylium* from asparagus spears, fern, or fern debris compared with two isolates of *S. botryosum* from lettuce and alfalfa

Isolate source	Isolates (no.)	Mean conidial dimensions: Length (μm)	Width (μm)	Length/width ratio	Shape	Lateral septal constrictions (no.)	Longitudinal septa (no.)	Transverse septa (no.)	Surface ornamentation	Pigmentation	Species
California asparagus	24	30(19–47)	14(9–22)	2.2	Oblong to broadly oval	1–3	1–2(–3)	2–7(–8)	Verrucose	Golden brown to olive brown	*S. vesicarium*
lettuce	1	29(22–34)	16(13–19)	1.8	Oval to oblong	1	1–2	3–5(–6)	Verrucose	Golden brown to olive brown	*S. botryosum*
alfalfa	1	32(28–47)	18(16–25)	1.8	Oval to oblong	1–2	1–2(–3)	3–5(–6)	Verrucose	Golden brown to olive brown	*S. botryosum*
Washington asparagus	3	31(23–46)	15(13–19)	2.2	Oblong to broadly oval	1–3	1–2(–3)	3–6(–7)	Verrucose	Golden brown to olive brown	*S. vesicarium*
Switzerland asparagus	3	34(25–47)	14(13–22)	2.4	Oblong to broadly oval	1–3	1–2(–3)	3–7(–8)	Verrucose	Golden brown to olive brown	*S. vesicarium*
England asparagus	2	38(28–56)	15(9–19)	2.8	Oblong	1–3	1–2	3–10	Verrucose	Golden brown to olive brown	*S. majusculum*

conidia shown in a photograph in Suzui's paper indicated that the pathogen in Japan may have been misidentified and is *S. vesicarium* rather than *S. botryosum*. It is also possible that *S. vesicarium* rather than *S. botryosum* is the cause of Stemphylium leaf spot of asparagus in Switzerland (D. Gindrat, *personal communication*).

S. vesicarium was isolated from asparagus fields in all of the main asparagus production areas in California (Fig. 3) except in the desert areas in the Imperial and Coachella valleys. Attempts to isolate *S. vescarium* in these two areas were unsuccessful, even though sampling was done during periods of prolonged wet weather. In the majority of fields sampled in desert areas, fern growth from the previous summer had been burned. This is an uncommon practice in other asparagus production areas of the state and may have helped to control the disease, as demonstrated in New Zealand (S. Menzies, *personal communication*).

Field observations. The level of disease at each harvest date was compared with the rainfall, evaporation, and temperature data for 1983 and 1984, respectively (Figs. 4 and 5, Table 2). There was more disease during the wet harvest season in 1983 than the relatively dry harvest season in 1984. Disease was most severe (as indicated by a short distance from the tip of each spear to the first lesion and high DSI) during the early weeks of harvest in both years. Disease severity was positively correlated with rainfall and the difference between rainfall and evaporation, but negatively correlated with evaporation (Table 2). That is, there was an increase in disease after periods of heavy rainfall when minimum and maximum air temperatures were between 0 and 20 C, respectively. Little, if any, wind movement of wet soil or sand was observed before these periods. In 1984, application of irrigation water through overhead sprinklers to one plot (Fig. 5B) resulted in increased disease, especially after the first applications (25 mm) on 6 and 9 March. Further applications of 10 mm on 28 March and 25 mm on 8 April resulted in only a slight increase in disease. Disease severity was more closely correlated with the difference between rainfall and evaporation than rainfall alone, indicating that the duration of wetness was more important than the amount of rain.

TABLE 2. Correlation coefficients between rainfall, evaporation, and the difference between rainfall and evaporation and disease severity (DSI) and mean length from the tip of each spear to the first lesion at Davis in 1983 and 1984

	1983		1984	
	DSI	Length from tip to first lesion	DSI	Length from tip to first lesion
Rainfall	0.567**[a]	−0.600***	0.353	−0.656**
Evaporation	−0.720***	0.726***	−0.349	0.740***
Rainfall-evaporation	0.718***	−0.746***	0.423	−0.859***

[a] Asterisks ** and *** indicate significance at $P > 0.01$ and $P > 0.001$, respectively.

Previous work showed that water was essential for rupture of asci, discharge, and germination of ascospores of *P. herbarum* (1) and *P. lycopersici* (16). It seems likely that periods of rain would result in release of ascospores of *Pleospora* from pseudothecia on asparagus debris close to growing spears. Because ascospores are forcibly discharged (1,20), they may be carried by wind and impacted on the windward side of spears.

Reproduction of symptoms. No disease symptoms developed on unwounded spears inoculated with conidia of *S. vesicarium* and placed on moist paper towels in a plastic container, consistent with an earlier report (11). However within 5–7 days after inoculation, many lesions developed on excised spears that had been kept with their bases in water or on spears of plants growing in U.C. mix. No symptoms developed on spears misted with sterile distilled water or inoculated with conidia of *S. vesicarium* but not covered with plastic bags. Symptoms developed on inoculated spears that had been kept under plastic bags for as little as 24 hr, although significantly ($P = 0.05$) more lesions developed on spears kept

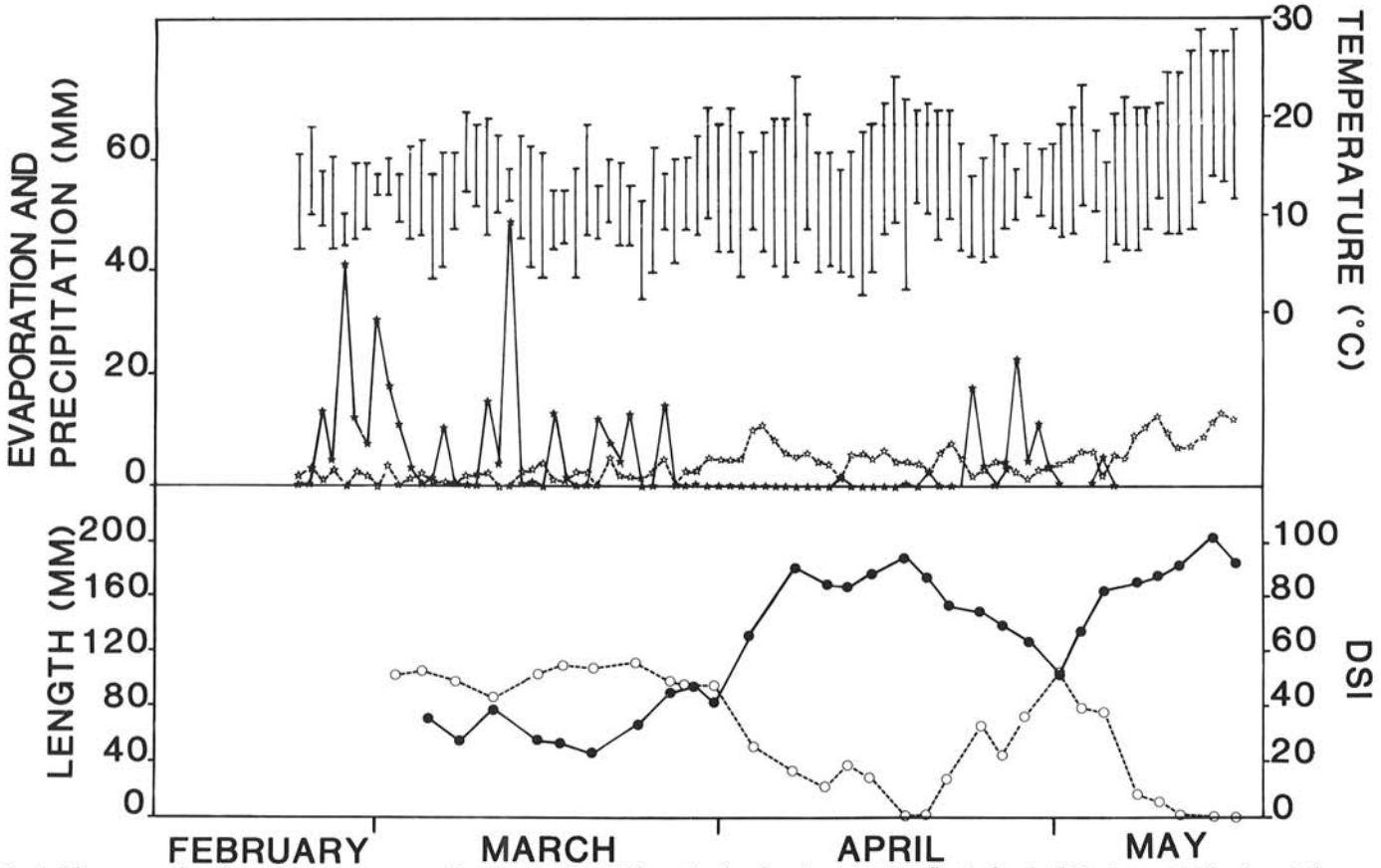

Fig. 4. Disease severity of purple spot as measured by the mean length from the tip of each spear to the first lesion (solid bullets, solid lines), and disease severity index (DSI) (open circle, dashed lines). Rainfall (solid stars, solid lines), evaporation (open stars, dashed lines), and minimum and maximum air temperature (vertical bars) data taken at Davis in 1983.

under bags for 48–72 hr. Isolations from such lesions gave pure cultures of *S. vesicarium*. These results demonstrated that disease can be caused by *S. vesicarium* on asparagus spears without prior wounding of the spears. This corresponds well with field observations where disease occurred after periods of wet weather, when little opportunity existed for sand-blasting of spears. The earlier reported observation that more lesions occur on one side of the spear than on the other (11) was probably a result of wind movement of ascospore inoculum and impaction of spores on the windward side of spears rather than sand-blasting. However, recent work (10) has shown that infections are more numerous and occur at shorter wetting durations on wounded than on nonwounded asparagus plants.

Lesions developed on spears surrounded with fern debris and covered with plastic bags for 24 hr. Disease severity increased significantly ($P = 0.05$) as time under plastic bags was increased to 72 hr. Isolations from diseased tissue gave pure cultures of *S. vesicarium*. No disease developed on spears misted with sterile distilled water for 72 hr in the absence of debris. These experiments and the results of pathogenicity tests of isolates from pseudothecia on fern debris indicated that dried fern stalks from the previous summer's fern growth were a principal source of ascospore inoculum for purple spot on spears in the spring.

Scanning electron microscopy. Germ tubes from ascospores (Fig. 6E) or conidia (Fig. 6A–D) penetrated the spears exclusively through stomata. No direct penetration was observed. This also has been reported for *S. botryosum* on alfalfa (3) and *S. loti* on bird's-foot trefoil (4). Epidermal cells collapsed around the point of penetration to form a slightly sunken lesion similar to that described by Lacy (11). Occasionally, germ tubes branched immediately over (Fig. 6B and C) or as far as 22 μm away from a stoma (Fig. 6D). Such branching may indicate a response of germ

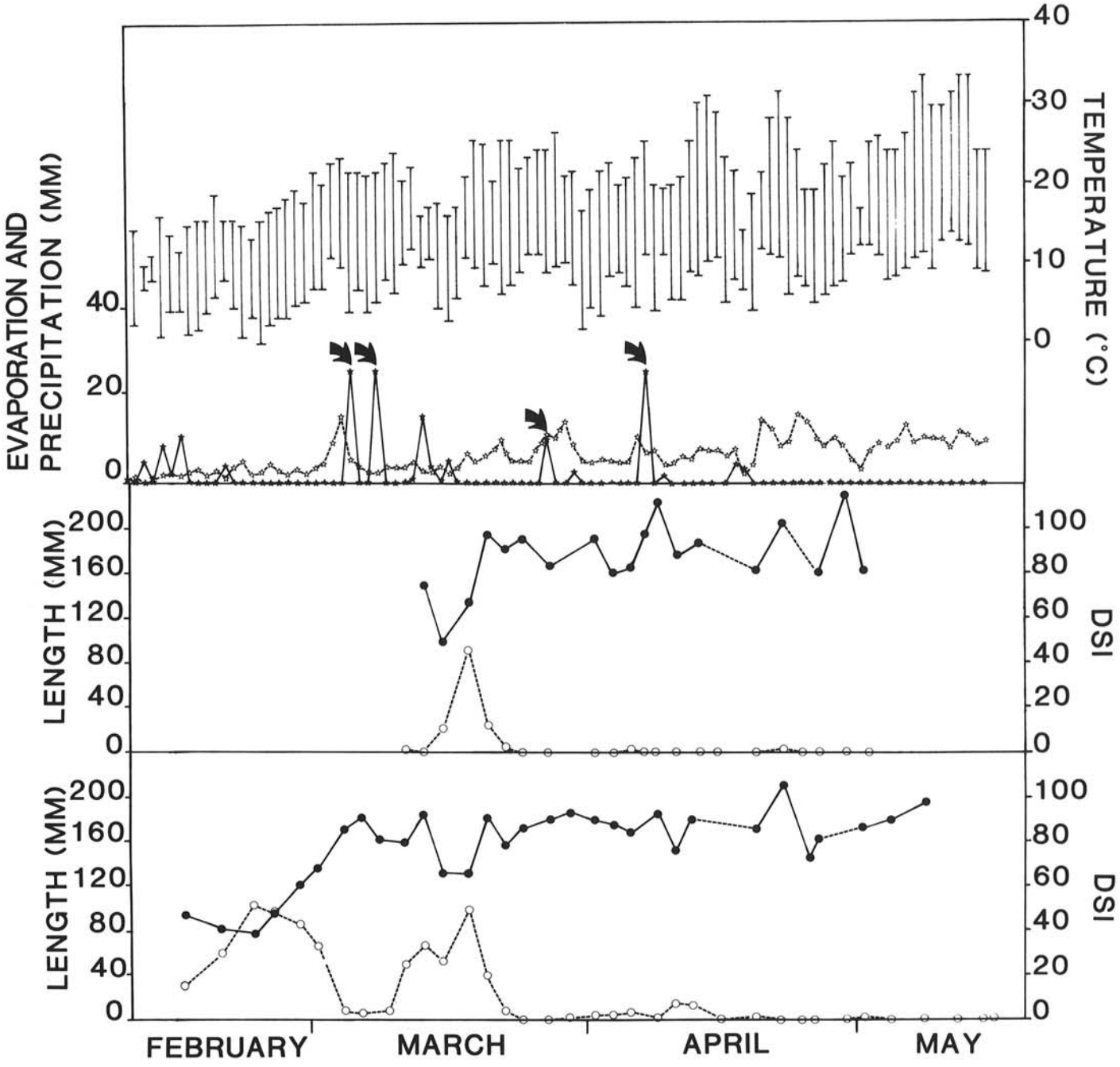

Fig. 5. Disease severity of purple spot as measured by the mean length from the tip of each spear to the first lesion (solid bullets, solid lines), and disease severity index (DSI) (open circles, dashed lines). Rainfall (solid stars, solid lines), evaporation (open stars, dashed lines), and maximum and minimum air temperature (vertical bars) data taken at Davis in 1984. (Solid bullets, dashed lines) indicates periods when no lesions were observed on the spears. Additional irrigation water was applied to plot B at points indicated by an arrow on the precipitation graph.

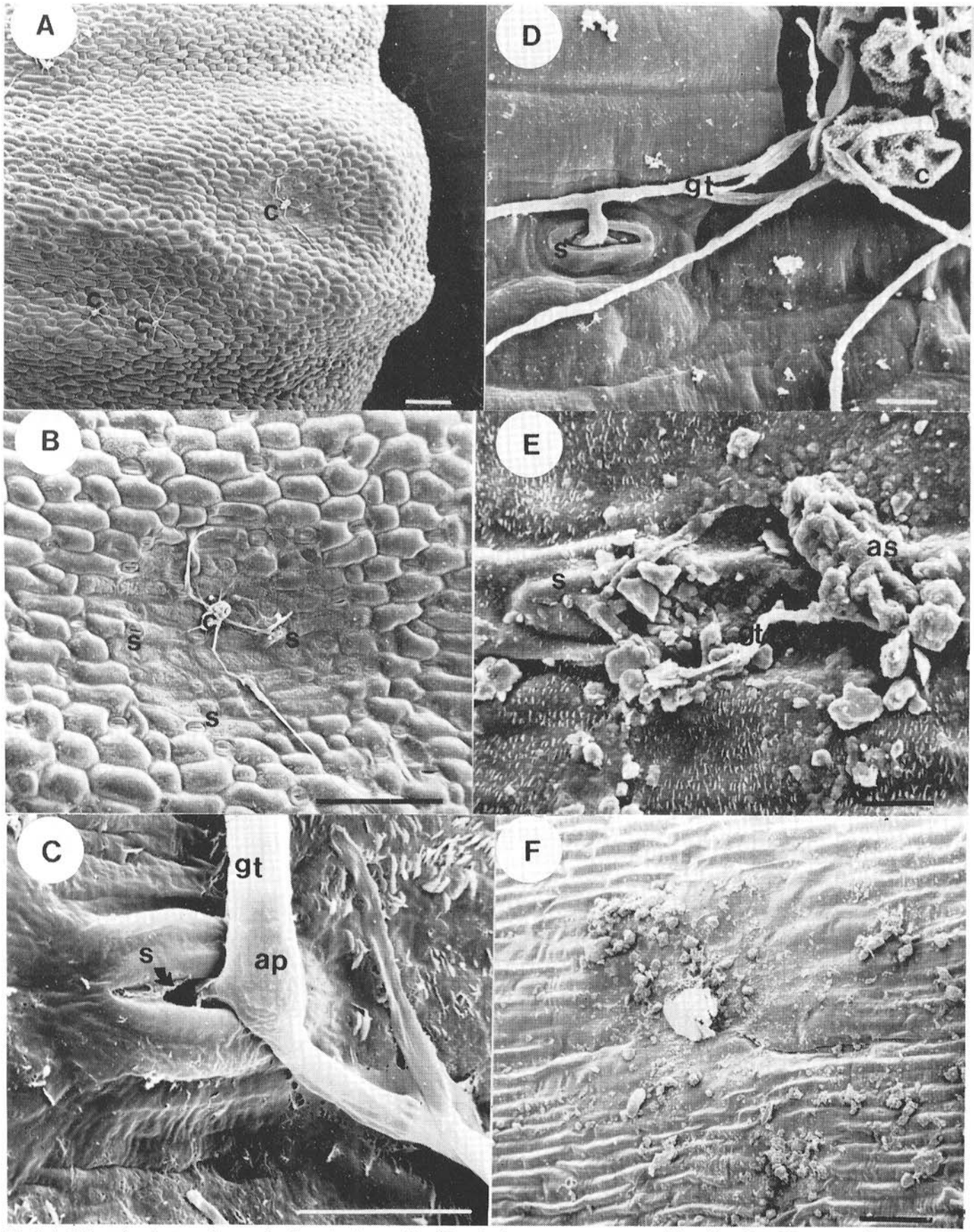

Fig. 6. A–D, Scanning electron micrographs of germinating conidia of *S. vesicarium* on asparagus spears of the cultivar U.C. 157 grown and inoculated in a growth cabinet. **E,** Penetration of germ tubes from conidia or ascospores on field-grown spears was exclusively through stomata. Epidermal cells collapsed around the point of penetration resulting in the formation of typical elliptical, slightly sunken lesions with a purple margin and brown center. **F,** Purple lesions were occasionally observed on spears in the absence of any signs of the pathogen and were probably the result of physical damage caused by impacting sand or soil particles. ap = appressorium, as = ascospore, c = conidium, gt = germ tube, and s = stoma. Scale bar in C–E = 10 μm, in A,B,F = 100 μm.

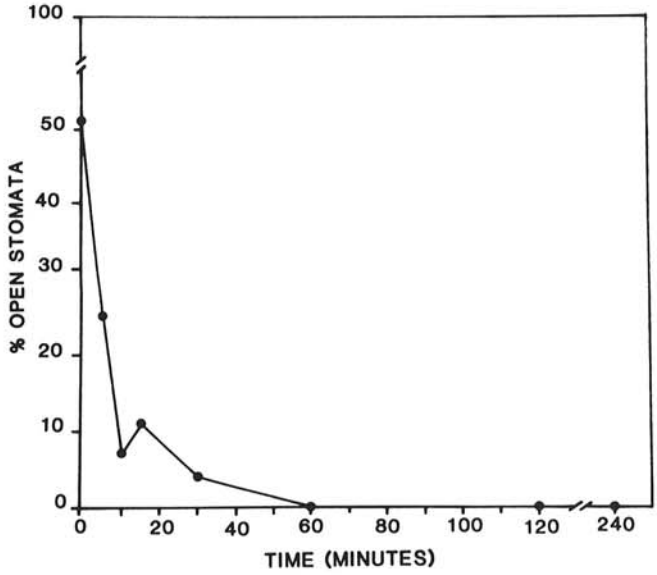

Fig. 7. Percentage of stomata that were open at various times after harvest of field-grown asparagus spears, cultivar U.C. 157.

tubes to open stomata. In almost all cases where penetration had occurred, germ tube growth was in the direction of the closest stoma. Whether this is a response to a physical or chemical stimulus is unknown. On inoculated spears that had no symptoms 14 days after inoculation, germ tubes were often observed to grow over the top of apparently closed stomata without appressorium formation. Small purple lesions that were associated with collapsed epidermal cells were occasionally observed on spears in the absence of any signs of the pathogen (Fig. 6F). *Stemphylium* was never isolated from these lesions and they probably developed in response to physical damage caused by impacting sand or soil particles.

Epidermal strips from harvested spears showed that stomata started to close 5 min after harvest, and that all stomata had closed within 1 hr after harvest (Fig. 7). Because penetration of asparagus spears by germinating conidia and ascospores of *Stemphylium*/*Pleospora* is through open stomata, failure to reproduce the disease on excised spears would be expected since stomatal closure starts soon after harvest. Wounding of excised spears before inoculation as done by several workers (5,11) probably provides an alternate portal of entry for the pathogen but has led to the incorrect conclusion that physical damage is requisite for disease development. Relief of water stress by placing the lower ends of excised spears under water immediately after harvest and before inoculation prevented stomata closure and allowed penetration of germ tubes.

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