Pod Rot of Dry Peas Due to Infection by Ascospores of *Sclerotinia sclerotiorum*

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ABSTRACT

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Dry peas (*Pisum sativum*) were artificially inoculated with airborne ascospores of *Sclerotinia sclerotiorum* over an 8-day period at the pod development stage. Pod rot lesions at the flower end of pods (basal rot) developed in 71.8% of pods. Lesion incidence at the distal end of pods (end rot) averaged 17.8%, whereas that on other parts of the pod tissues averaged 11.8%. The scanning electron microscopy studies illustrated that the high incidence of basal rot was attributable to close contact between pod tissues and the stamens, which provided substrate for germination of pathogen ascospores, subsequent ramification of mycelia, and the creation of specific infection sites for the basal rot pathogen. Mycelia on anthers and filaments spread onto the pod surface and developed infection cushions consisting of compact, dichotomously branched hyphae that initiated the penetration of pod tissues. Lesions of end rot appeared most frequently on green pod tissue at the base of the senescent style. Pollen grains often were invaded by *S. sclerotiorum*. The role of anthers, pollen grains, filaments, and styles in the development of Sclerotinia pod rot in dry peas is discussed.

Additional keywords: ascospore infection

Sclerotinia stem rot and pod rot of peas (*Pisum sativum* L.) caused by *Sclerotinia sclerotiorum* (Lib.) de Bary has been reported in Nova Scotia (11), Ontario (29), Manitoba (23), Saskatchewan (2,17), and Alberta (27), Canada; Colorado (22), Idaho (5), Michigan (15), Washington, and Oregon (9); New Zealand; Morocco; the Netherlands; Bermuda; Brazil; Argentina; and Scotland (8). The disease occurs most frequently during the later stages of plant growth, from flowering to maturity (5,17,27). High humidity and dense canopy are conducive to severe outbreaks of the disease (8,22). In western Canada, disease incidence has been reported as light (2,23,27) to moderately severe (17). However, severe outbreaks of the disease have been reported in Nova Scotia (60% pod blight and rot) (11) and in northern Colorado (only parts of a pea field were harvested) (22).

Airborne ascospores are the primary source of inoculum for Sclerotinia infection of pea (20), bean (*Phaseolus vulgaris* L.) (1,4), tomato (*Lycopersicon esculentum* Mill.) (25), safflower (*Carthamus tinctorius* L.) (19), canola (*Brassica campestris* L. and *B. napus* L.) (7), and soybean (*Glycine max* (L.) Merr.) (28). A suitable nutrient base such as senescent flowers (4,18,21,25,28) or injured leaves (1,18,24) is required for ascospore germination and subsequent penetration of host tissues. Lumsden and Dow (16) reported that infection of bean tissues by *S. sclerotiorum* occurred by formation of an infection cushion and development of dichotomously branched, penetration hyphae or appressoria (24). Purdy (24) observed that both mechanical contact and a carbon source were required for the formation of appressoria.

Although Sclerotinia pod rot of peas has been found under natural field conditions, there are no reports on the etiology of the disease in this crop. The objective of this study was to investigate the role of pea stamens and stylar tissues in the induction of pod rot by airborne ascospores of *S. sclerotiorum*. The role of senescent petals is excluded from this investigation because most of these petals drop readily to the ground or onto other parts of the plant and they rarely remain attached to pea pods.

MATERIALS AND METHODS

Seeds of dry peas, cultivar SS 2, were sown in soil in plastic pots (15 cm diameter). The pots were kept in a growth cabinet at 22 C under a 16-hr photoperiod with light intensity at 158 $\mu E \cdot s^{-1} \cdot m^{-2}$ maintained by a mix of incandescent and fluorescent lamps. The emerged seedlings were thinned to two plants per pot. Plants were watered daily and were inoculated with *S. sclerotiorum* after formation of the third or fourth pod (approximately 6 wk after seeding).

Four experiments were conducted using 16 pots for experiment 1 and 24 pots each for experiments 2–4. Half of the plants in each experiment were inoculated with *S. sclerotiorum* and the other half were left as untreated controls. Sclerotia of isolate sun-87 were harvested from cultures grown on potato-dextrose agar at 10 C for 8 wk and incubated on moist sand in petri dishes (6 cm diameter) under light at 20 C for 2–3 wk to produce mature apothecia (13). For experiments 1 and 2, a petri dish containing 10 sclerotia with mature apothecia on moist sand (13) was placed on the soil surface of the pot and, for experiments 3 and 4, six sclerotia with apothecia were transplanted to the soil surface in each pot (Fig. 1). The plants in each pot were covered with a transparent plastic bag that acted as a moisture chamber. Plants were watered daily through a saucer at the base of each pot. The bag was opened for 5–10 sec each day during the first 2 days of bagging to promote spore discharge and allow the spore cloud to mix with air in the chamber and land on plant tissues at random. Eight days later, the pods on each plant were examined for the development of lesions caused by *S. sclerotiorum*. In each experiment, most of the senescent petals were detached from the pea pods either by natural causes or by disturbance of plants during the inoculation procedure, which involved moving pots and staking and covering plants. Some senescent petals remained attached to the pod and provided substrates for ascospore infection on pod tissues, and such petal-mediated pod rots were excluded from the calculations of disease incidence. This was done because the senescent petals were readily detachable from pea pods. Thus, the petal-mediated infections were not necessarily confined to pea pods.

To confirm that pods were infected with *S. sclerotiorum*, pods with small or large lesions from the first two experiments were surface-sterilized in 90% ethanol for 90 sec, placed on moist filter

Fig. 1. Method of inoculation of pea plants with *S. sclerotiorum* in a pot by placing sclerotia with mature apothecia (arrows) on the soil surface. Scale bar = 20 mm.

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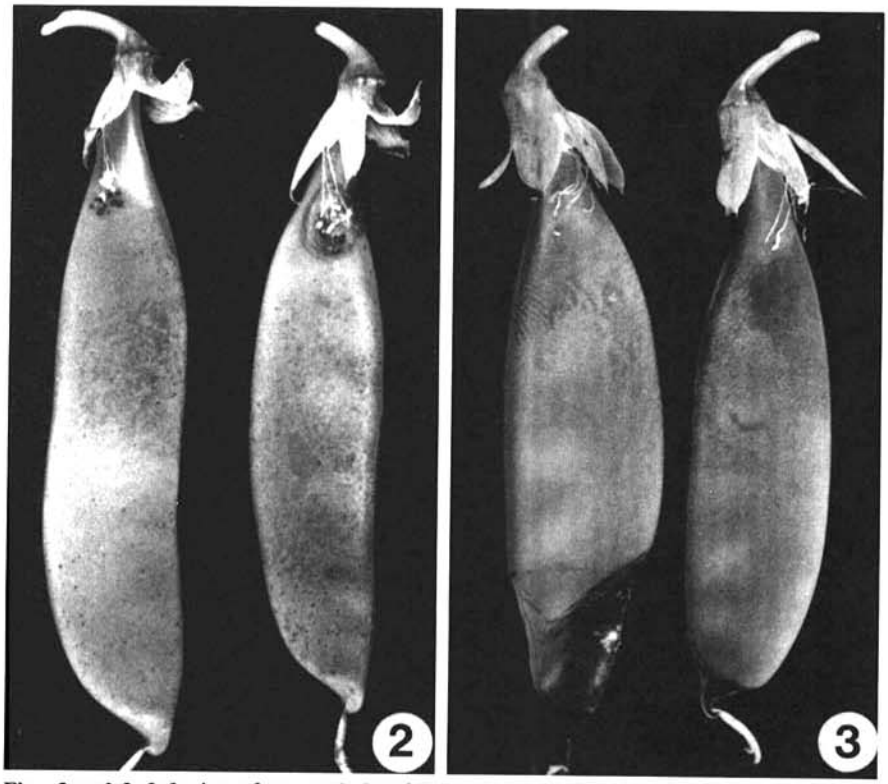
paper in petri dishes, incubated at room temperature (20 ± 2 C) for 7 days, and then examined for the development of colonies and formation of sclerotia on the diseased pods.

Pea pods with small (≤1 mm) to large (≥5 mm) lesions were used for study by scanning electron microscopy. Pod tissues from lesioned areas in direct contact with anthers or filaments as well as nonlesioned areas were excised. These tissues were immersed in 2% glutaraldehyde fixative in 0.05 M sodium phosphate buffer, pH 7.0, overnight at 4 C. After buffer rinses, the specimens were dehydrated in a graded series of ethanol and critical-point dried (Polaron E3100) with liquid carbon dioxide as the transitional fluid. The material was adhered onto aluminum specimen mounts with colloidal silver paste, air-dried overnight, and sputter-coated (Denton Vacuum Desk-1, Cherry Hill, NJ) with gold (approximately 15 nm thickness). The specimens were examined and photographed on a Hitachi S-570 scanning electron microscope.

RESULTS

Airborne ascospores naturally discharged from apothecia of *S. sclerotiorum* on the soil surface (Fig. 1) infected pea plants, and developing lesions were visible 4–5 days after incubation in the plastic bag. After an 8-day incubation, dark brown to grayish brown lesions, ranging from <1 mm to >5 mm, were observed on pods (Figs. 2 and 3), stems, leaves, and tendrils. All of the lesions were associated either with injured tissues, such as broken tendrils, or direct contact with senescent tissues, such as stamens or petals. No infection was observed in the untreated control plants (Table 1).



Figs. 2 and 3. Infection of pea pods by airborne ascospores of *S. sclerotiorum* resulting in the development of lesions **(2)** at the base and **(3)** distal end of the pod. Note the association of stamens with the initiation of basal lesions (Fig. 2).

Table 1. Location of pod rot lesions of dry peas (cv. SS 2) from infection by airborne ascospores of *Sclerotinia sclerotiorum*

		Frequency (%)[a]			
Treatment	Type of rot	Experiment 1	Experiment 2	Experiment 3	Experiment 4
Inoculated[b]	Basal rot	67.4 (39)	76.9 (54)	58.1 (57)	85.1 (53)
	End rot	···	24.5	14.0	15.1
	Other pod rot	···	···	···	11.8
Control	Basal rot	0 (38)	0 (49)	0 (54)	0 (49)
	End rot	0	0	0	0
	Other pod rot	0	0	0	0

[a]The number of pods in the treatments of each experiment is given in parentheses.
[b]Pea plants with young pods were inoculated with airborne ascospores of *S. sclerotiorum* for 8 days in a moist chamber. Pod rot induced by contact with senescent petals in each experiment was excluded.

Pod rots were initiated by infection at the base (basal rot) (Fig. 2), distal end (end rot) (Fig. 3), and/or other parts of the pod. In the four inoculation experiments, the incidence of basal rot was high, averaging 71.8% infected pods (Table 1). Compared with basal rot in each experiment, end rot incidence was low, averaging 17.8% infected pods. The frequency of lesions on other parts of the pod also was low (11.8%) (Table 1). When pods with visible lesions were incubated in moist petri dishes for 1 wk, all produced white mycelial mats with sclerotial primordia or black sclerotia. Pods from control plants remained healthy.

Development of basal pod rot always was induced by direct contact between senescent stamens and young pod tissue (Fig. 2). The 10 stamens in each flower remain firmly attached to the pod base throughout the entire pod formation and seed development stage. Under moist conditions, ascospores of *S. sclerotiorum* landed on the stamens, germinated, and produced threadlike running hyphae that colonized the filament and anther (Fig. 4). The running hyphae spread from the stamen to the pod with formation of numerous infection cushions on the pod surface (Fig. 5). Simple (Fig. 6) to complex (Fig. 7) infection cushions were formed by repeated dichotomous branching of hyphae. Penetration occurred at the bottom layer of hyphal cells of each infection cushion, which was in firm contact with pod tissues (Figs. 6 and 7). When the stamen in direct contact with pod tissue was examined under a stereomicroscope, numerous tiny brown lesions, each with an infection cushion at its center, were observed scattered on the pod near the filament or anther. The small lesions often coalesced to form larger lesions and eventually basal rot. In addition to colonizing stamens, hyphae of *S. sclerotiorum* on an anther often invaded pollen grains by direct penetration through the pollen walls (Figs. 8 and 9) and/or germ pore.

The first sign of pod end rot was the appearance of a light-brown to brown discoloration at the base of the style and a small water-soaked lesion on green tissue at the end of the pod. Under humid conditions, the lesion expanded rapidly from the distal end toward the base of the pod (Fig. 3). Lesions that developed on other parts of the pod always resulted from direct contact with senescent flower petals or fragments of anthers and filaments.

DISCUSSION

This study of pod rot of dry peas confirms previous reports (4,18,21,25,28) that exogenous nutrients are required for germination of ascospores of *S. sclero-*

tiorum and subsequent infection of host tissues. The senescent stamens and style remained firmly attached to the pea pod during the seed development stage. Heavy colonization of filaments and anthers by mycelia of *S. sclerotiorum* suggests that these tissues provide adequate nutrients for germination of ascospores and growth of hyphae and serve as initial infection sites for basal rot. Results indicate that, under the same inoculum pressure, the incidence of basal rot is higher than that of end rot. This is perhaps because each pod has numerous stamens but only one style that can be precolonized by *S. sclerotiorum* before penetration of the pod tissue.

Previous studies have shown that anthers (26) and pollen (3,6,10,30) stimulate spore germination of fungal pathogens and enhance lesion development on plants. Hartill (10) found that 50% of the ascospores of *S. sclerotiorum* placed on leaf disks of tobacco (*Nicotiana tabacum* L.) germinated in the presence of tobacco pollen, but relatively few spores germinated without pollen. Sutton and Deverall (28) found that pollen grains of bean, tobacco, or rye (*Secale cereale* L.) stimulated growth of germinating ascospores of *S. sclerotiorum* but did not stimulate infection of bean; others (3,6,30) reported that infection of fungal pathogens on hosts in the presence of pollen was much heavier than in the absence of pollen. Further evidence of the epidemiological significance of pollen is the fact that a pathogen can attack its host's pollen grains, as was observed for *S. sclerotiorum* on dry peas in this study, and *Verticillium albo-atrum* Reinke & Berthier on alfalfa (*Medicago sativa* L.) in other studies (12,14). Pollen grains may be important for development of pod rot in dry peas because they provide substrate for mycelial growth of *S. sclerotiorum* before penetration of green pod tissue.

Lumsden and Dow (16) reported that infection of bean by *S. sclerotiorum* was achieved by the firm contact of infection cushions of the pathogen on host tissues. This infection process is identical to that of the basal pod rot of dry peas observed in this study. Because of the proximity of senescent stamens to the pea pod, all filaments and anthers are potential substrates for the ascospores that cause basal pod rot. Senescent petals of dry peas are also a good substrate for precolonization by *S. sclerotiorum*, as has been reported for other crops (18,21,25,28). However, senescent petals are easily detached from pea pods. Thus, the sites of petal-mediated infections are not always on the pea pods and, depending on the point of chance contact, senescent petals may induce lesion development on stems, leaves, or tendrils.

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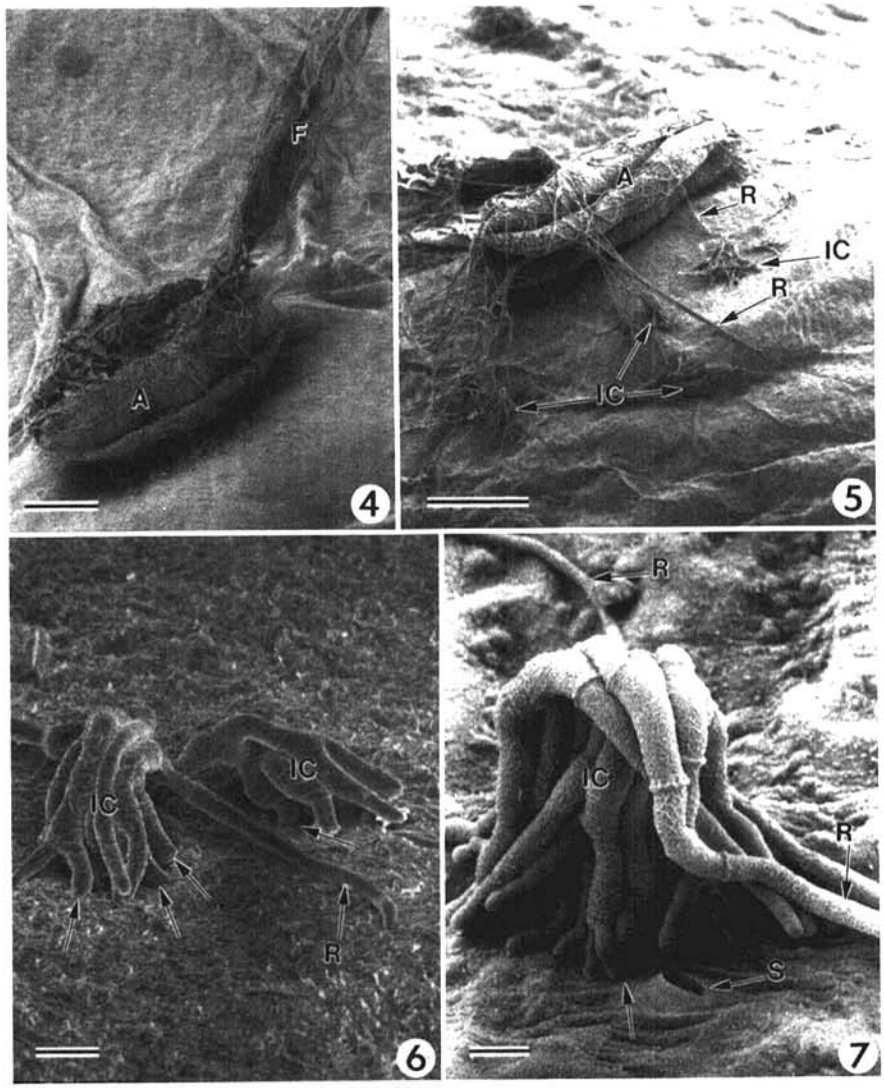
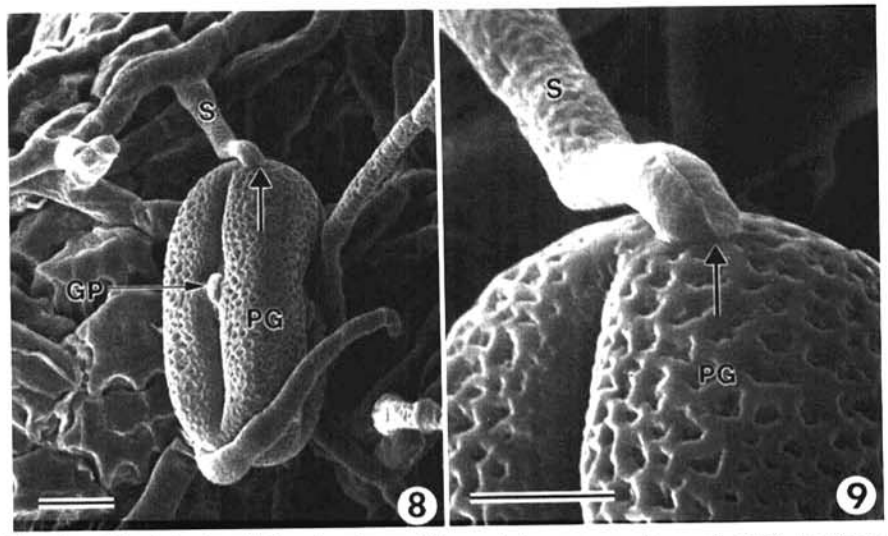


Figs. 4–7. (4) Early stage of colonization of a pea stamen by airborne ascospores showing intense mycelial growth on the filament (F) and anther (A). Scale bar = 200 μm. **(5)** Running hyphae (R) of *S. sclerotiorum* spreading from the anther (A) to form numerous infection cushions (IC) on the pod surface. Scale bar = 200 μm. **(6)** Two simple and **(7)** one complex infection cushion (IC) produced from the running hypha (R) of *S. sclerotiorum*. Note that the infection cushions are formed by dichotomously branched hyphae and some of the hyphal tips appear to be penetrating the pod tissues (arrows). S = stoma. Scale bar = 10 μm.



Figs. 8 and 9. A hypha of *S. sclerotiorum* (S) penetrates a pea pollen grain (PG) by direct penetration of the pollen grain wall (arrow). GP = equatorial germ pore. **(8)** Scale bar = 10 μm; **(9)** Scale bar = 5 μm.

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