Localized Infections of Siliques and Seed of Cabbage by *Phoma lingam*

J. M. BONMAN, Graduate Research Assistant, and R. L. GABRIELSON, Plant Pathologist, Western Washington Research and Extension Center, Puyallup 98371

ABSTRACT

Bonman, J. M., and Gabrielson, R. L. 1981. Localized infections of siliques and seed of cabbage by *Phoma lingam*. Plant Disease 65:868-869.

Cabbage siliques and seed, both naturally infected with *Phoma lingam*, were found in a field in the cabbage seed growing region of the Pacific Northwest. All silique infections were localized, indicating that infection did not progress systemically from basal crown cankers. Infected siliques had internal blackening of the locular walls or the suture, usually confined to the stigmatic end. Few showed visible external signs or symptoms of infection. The difficulty of finding blackleg in Pacific Northwest seed fields suggests that a direct seed test would more reliably predict disease potential of seed lots.

In cabbage seed fields, an important part of the biology of the blackleg organism, *Phoma lingam* (Tode ex Fr.) Desm., is the mode of seed infection. From field observations, Clayton (4) concluded that conidia from leaf or branch lesions are dispersed to the siliques by splashing water. From there, the conidia germinate and eventually infect maturing seed. In the Pacific Northwest, *P. lingam* has occasionally been found on cabbage seed plants, and infected seed lots have been produced (5,11); however, infected siliques have not previously been observed here (10). This has led to speculation that the pathogen infects seed systemically.

Our paper expands on a preliminary report (2) describing the symptoms and signs of a natural, localized *P. lingam* infection of cabbage siliques and seed. We have also tested the ability of the fungus to infect seed systemically.

MATERIALS AND METHODS

Natural infection. A 5-ha cabbage seed field near Junction City, OR, containing plants infected with *P. lingam* was used in the study. In July, samples of 25 stem bases, 100 overwintered leaves, and 100 siliques were selected at random from each of nine equal divisions of the field. These samples were examined in the field for *P. lingam* infection. In addition, whole plants with stem cankers were removed for further laboratory study.

To identify siliques carrying infected seed, we removed the seeds from all siliques on two plants with blackleg cankers and assayed them for the presence of *P. lingam*, using the procedure recommended by the International Seed Testing Association (7). The location of each silique on the plant was recorded. Because infected seed came only from siliques with internal blackening from these two plants, only internally blackened siliques and their supporting structures were chosen for further isolations from nine additional cankered plants.

Alternaria brassicicola (Schw.) Wiltsh. was sporulating on many severely blackened siliques. To determine whether Alternaria disease could mask *P. lingam* infection assays from these siliques, we compared them with assays from siliques having no *A. brassicicola* sporulation.

We tested for the presence of the pathogen in tissues between crown cankers and infected siliques. Isolations were attempted from the stigmatic and pedicellar ends of each silique containing infected seed, the pedicel, and the supporting branch 5 and 20 cm below the point of silique attachment. Excised tissue was dipped in 70% ethyl alcohol, flamed momentarily, and plated on V-8 juice agar with rose bengal at 40 μg/ml and streptomycin sulfate added at 100 μg/ml (8).

In October, a postharvest seed sample from this field obtained from the seed company was also assayed for *P. lingam*.

Thirty-eight mass isolates from seed, siliques, and crown cankers from the plants described above were tested for virulence as described previously (3).

Artificial inoculation. Bolting cabbage seed plants of the variety Stonehead, grown in the greenhouse, were artificially inoculated in a test of the pathogen's ability to infect seed systemically. Six plants were injected at the stem base with 0.25 ml of a suspension containing 10^7 conidia per milliliter, three with a composite of weakly virulent isolates, and three with a highly virulent composite. Each composite was made from five isolates. Two check plants were inoculated with sterile water. At flowering, the plants were hand pollinated. One hundred days after inoculation, seeds in 10 siliques from each plant were tested for *P. lingam* infection. Isolations were also attempted from the stem tissues to confirm that infection had taken place.

RESULTS

Natural infection. No lesions were observed on siliques in the field, although 24% of the plants had crown cankers and 62% of the overwintered rosette leaves had blackleg lesions. In the seed sample from the commercial harvest, 0.25% was infected with *P. lingam*.

Laboratory examination of more than 10,000 siliques yielded 107 that contained infected seed. These 107 siliques had internal blackening varying from black streaks along a small portion of the locular suture (Fig. 1) to complete blackening of the locular walls. Most often, however, the necrosis encompassed only part of the silique, the rest remaining symptomless (Fig. 2). The fungus was isolated more often from the stigmatic end of siliques than from the pedicellar

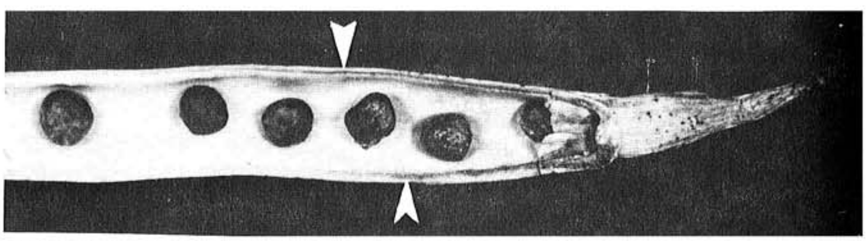

Fig. 1. Blackening of the locular suture of a cabbage silique naturally infected with *Phoma lingam*.

Present address of senior author: The Rockefeller Foundation, G.P.O. Box 2453, Bangkok, Thailand.

Scientific Paper 5716, Project 0189, of the College of Agriculture Research Center, Washington State University, Pullman 99164.

Supported in part by USDA/SEA-CR grant 516-15-120 and by a grant from the American Seed Research Foundation.

Accepted for publication 24 March 1981.

0191-2917/81/11086802/$03.00/0

end, and the most pronounced blackening was also usually found there (Table 1). No infected seeds were recovered from symptomless siliques. Seed samples from siliques severely blackened by *A. brassicicola* occasionally contained some seed infected with *P. lingam*.

Although siliques infected with *P. lingam* were blackened internally, external discoloration was slight. *P. lingam* pycnidia, however, were present on 89% of the infected siliques, but only 7% of these had easily discernible pycnidia (Table 2).

Isolations were attempted from 95 siliques containing infected seeds, and in each case *P. lingam* was isolated. However, the pathogen was only rarely isolated from the plant basipetally. *P. lingam* was recovered from only 9% of the fruit pedicels, and in no case was the fungus isolated from all points tested below an infected silique.

All of the isolates tested were weakly virulent.

Artificial inoculation. Although the pathogen was readily recovered from the bases of the inoculated plants, none of the seeds became infected.

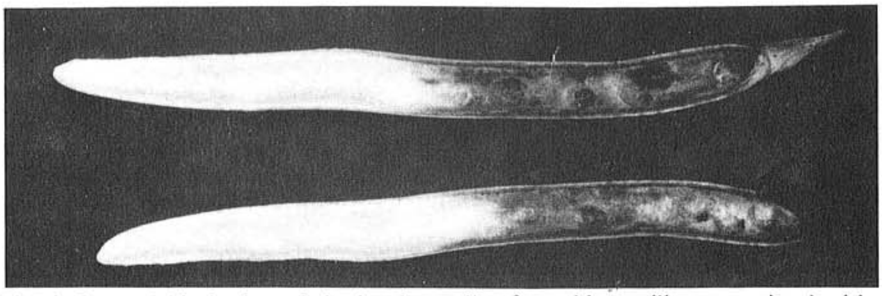

Fig. 2. Internal blackening of the locular walls of a cabbage silique associated with *Phoma lingam* infection.

Table 1. Frequency of internal blackening and *Phoma lingam* isolation at different locations on naturally infected cabbage siliques

Location	Internal blackening[y] (%)	*P. lingam* isolation[z] (%)
Stigmatic end only	85	71
Pedicellar end only	13	8
Both ends	2	21

[y]Total of 105 siliques.
[z]Total of 95 siliques.

Table 2. Percentage of surface area of naturally infected cabbage siliques showing *Phoma lingam* pycnidia

Pycnidia	Infected siliques[z] (%)
None present	11
On <1% of silique	59
On 1–5% of silique	22
On >5% of silique (easy to see)	7

[z]Total of 107 siliques.

DISCUSSION

The *P. lingam* infection patterns on cabbage plants in our field and greenhouse experiments suggest that *P. lingam* does not infect cabbage seed systemically. Field infections were localized on the plants, and isolation of the fungus from points below infected siliques was rare. This pattern indicates that infection did not progress systemically from basal crown cankers to siliques. Further, the predominance of stigmatic end infections would not be expected if the pathogen had grown upward from the base of the plant. This predominance could be caused by the early death and abscission of siliques infected through the pedicels, as observed by Henderson (6) and Gabrielson (*unpublished*) after field inoculations.

The difficulty of finding siliques infected with *P. lingam* in the seed producing regions of the Pacific Northwest is complicated by the lack of obvious external signs and symptoms, the presence of other fungi, and the generally low levels of *P. lingam* in the field (5). In this field study, the incidence of silique infection was low, and pycnidia on infected siliques were difficult to see. Although overwintered leaves and stem bases were often infected, only about 1% of the siliques examined and 0.25% of the commercially processed seed were infected with *P. lingam*. The low incidence of silique infection was probably caused by the little rainfall during fruit development, which is typical for the Pacific Northwest in June and July (10). Lack of rainfall reduced the chances of conidia being dispersed by splashing to the siliques from previously infected plant parts.

The siliques would logically have a much lower incidence of infection than either leaves or stems, which are present throughout the winter when rain can easily spread inoculum. The relatively dry summer climate in this region may also account for the limited production of pycnidia on infected siliques. We also noted that only the weakly virulent strain of the pathogen was isolated from the field, even from siliques with the most pycnidia. This strain sporulates less copiously on cabbage cotyledons than the highly virulent strain (3), as may also be true for cabbage siliques.

Other fungi can mask the pycnidia and internal blackening of *P. lingam* infection. For example, some seeds infected with *P. lingam* were found in siliques infected with *A. brassicicola*. *Mycosphaerella brassicicola* (Fr.) Lindau and *Peronospora parasitica* (Pers. ex Fr.) are also commonly found in Pacific Northwest crucifer seed crops. They can cause severe blackening of siliques and other plant parts (1,9).

In 6 yr of observation, *P. lingam* symptoms have rarely been seen in Pacific Northwest seed fields by us or by state inspectors. The field in this study was by far the most severely affected. During this time, however, a significant number of infected seed lots were found using the procedure recommended by the International Seed Testing Association (5,7). Some of the lots had levels of infection greater than 0.25%. Field inspections are not a sensitive method of ascertaining the disease producing potential of cabbage seed from the Pacific Northwest. Phytosanitary certificates are currently being issued based on field inspections for *P. lingam*. Basing certificates on a direct seed test (7), followed by a virulence test (3) of any *P. lingam* isolates from seed, would have far more validity.

LITERATURE CITED

1. Babadoost, M., and Gabrielson, R. L. 1979. Pathogens causing Alternaria disease of brassica seed crops in western Washington. Plant Dis. Rep. 63:815-820.
2. Bonman, J. M., and Gabrielson, R. L. 1979. Localized infection of siliques and seed of cabbage seed plants by *Phoma lingam*. (Abstr.) Phytopathology 69:1022.
3. Bonman, J. M., Gabrielson, R. L., Williams, P. H., and Delwiche, P. A. 1981. Virulence of *Phoma lingam* to Cabbage. Plant Dis. 65:865-867.
4. Clayton, E. E. 1928. Seed treatment for blackleg disease of crucifers. N. Y. Agric. Exp. Stn. Tech. Bull. 137.
5. Gabrielson, R. L., and Maguire, J. D. 1977. The biology and control of *Phoma lingam* in crucifer seed crops. Search 14:2-8.
6. Henderson, M. P. 1918. The blackleg disease of cabbage caused by *Phoma lingam* (Tode) Desmaz. Phytopathology 8:379-431.
7. Maguire, J. D., Gabrielson, R. L., Mulanax, M. W., and Russell, T. S. 1978. Factors affecting the sensitivity of 2,4-D assays of crucifer seed for *Phoma lingam*. Seed Sci. Tech. 6:915-924.
8. McGee, D. C., and Petrie, G. A. 1978. Variability of *Leptosphaeria maculans* in relation to blackleg of oilseed rape. Phytopathology 68:625-630.
9. Pound, G. S. 1946. Diseases of cabbage plants grown for seed in western Washington. Wash. Agric. Exp. Stn. Bull. 475. 27 pp.
10. Pound, G. S. 1947. Variability in *Phoma lingam*. J. Agric. Res. 75:113-133.
11. Pound, G. S., Cheo, P.-C., Calvert, O. H., and Raabe, R. D. 1951. Extent of transmission of certain cabbage pathogens by seed grown in western Washington. Phytopathology 41:820-828.