

Studies on the biology and control of *Ascochyta fabae* on faba bean¹

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The optimum temperature for radial growth of *Ascochyta fabae* mycelium on agar and for fungal infection of Erfordia faba bean (*Vicia faba*) was 20°C. *A. fabae* did not survive over winter in field plots in which infected plants had been ploughed down the previous year. Less than 3% of internally infected faba bean seed produced infected seedlings in greenhouse and field trials. In the field, seed treatment trials with captan, benomyl, and thiram did not significantly affect the amount of seedling or adult plant infection. Infection levels of 4% to 10% in field plots caused no significant loss in yield.

Can. Plant Dis. Surv. 57: 31-35. 1977

La température optimale pour la croissance radiale de *Ascochyta fabae* sur gélose et à l'infection fongique du cultivar de féverole Erfordia (*Vicia faba*) était de 20°C. *A. fabae* n'a pu survivre à l'hiver dans les parcelles dont les plants infectés avaient été enfouis d'année précédente. Moins de 3% des semences de féverole infectées (infection par la semence) ont produit des plantules infectées lors d'essais en serre et en plein champ. Des essais de traitement des semences au captane, benomyl et thirame n'ont pas influé significativement sur le taux d'infection des plantules ou des plants adultes en plein champ. Des taux de 4 à 10% en parcelles n'ont causé aucune baisse significative de rendement.

Two seed-borne diseases, chocolate spot caused by *Botrytis fabae* Sard. and leaf and pod spot caused by *Ascochyta fabae* Speg., were reported in Nova Scotia in 1970 (2). *A. fabae* caused higher incidences of seed infection and it was suggested that this organism might be a greater threat to faba bean production than the chocolate spot disease. In 1972, the acreage of faba bean increased dramatically as a new crop in Canada, and it was apparent that diseases known to cause damage in Europe should be investigated for their potential destructiveness in Canada.

The present work was initiated because of concern about the effects of leaf and pod spot on faba bean, since large quantities of seed were being imported from Europe for planting in Canada. A number of experiments were conducted: temperature effects on growth and infectivity of *A. fabae* and of the closely related fungus *Ascochyta pisi*, the cause of leaf and pod spot of pea (*Pisum sativum* L.); infection experiments with *A. fabae* and three *Ascochyta* pathogens of pea; survival of *A. fabae* in soil; fungicide seed treatments to control the seed-borne phase of the faba bean disease; and the relationship of seed infection to subsequent infection in the crop and to yield.

Methods and materials

Isolates of *A. fabae* were obtained from diseased seed samples submitted to Plant Products Division, Agriculture Canada, for examination. Isolates of *Ascochyta pisi*

Lib., *Ascochyta pinodes* L.K. Jones, and *Ascochyta pinodella* L.K. Jones (*Phoma medicaginis* var. *pinodella* (L.K. Jones) Boerema) were from stock collections maintained at the Ottawa Research Station.

The effect of temperature on radial growth of mycelium of *A. fabae* and *A. pisi* were studied using agar discs (6 mm) from petri dish cultures of the fungi placed on the center of pea, faba bean, and Difco malt agars in 90 mm petri dishes. The pea and faba bean media were prepared as follows: 360 g of seed in 2.2 litres of water were autoclaved for 2 h at 121°C; and the resulting mash was strained through two layers of cheesecloth, the volume made up to 3 liters and 45 g of Difco agar added. The medium was dispensed into 1 litre flasks and sterilized 20 min at 121°C. The colonies were incubated in darkness at constant temperatures (10°C - 35°C ± 1°C, 5° intervals) with 5 replicates per temperature. The radial growth (mm) was recorded daily for 14 days.

Three methods of inoculating 16-day-old Erfordia faba bean plants with an aqueous spore suspension (0.5 X 10⁶ spores/ml) of *A. fabae* containing two drops of Tween 80 per 100 ml were compared at temperatures of 15°, 20°, and 25°C in growth chambers using a 16-h day and a relative humidity of 95-100%. Spore suspension was sprayed to run-off on 1) unwounded leaves, 2) leaves abraded with no. 600 emery paper, and 3) plants wounded by the addition of no. 600 (medium sharp) silicon carbide to the spore suspension. Disease severity on leaves and stems was determined 14 days after inoculation using a method described previously (4) to rate disease severity of *A. pisi* on peas. Similarly the infectivity of *A. fabae* on improved Laxton's Progress peas and of *A. pinodella*, *A. pinodes*, and *A.*

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A. pisi on *Erfordia faba* bean was studied with 11-day-old seedlings.

Soil samples from field plots that had been planted to infected seed in 1974 and 1975 were assayed to determine if the fungus could overwinter at Ottawa. These plots contained debris from infected plants that had been ploughed into the soil. Composite soil samples were taken to a depth of 5 cm from each of 16 plots in the fall of 1974 and in the spring and fall of 1975. The samples were heated in a forced-air oven at $50 \pm 1^\circ\text{C}$ for 12 h. Surviving propagules were isolated from serial dilutions of the soil samples on rose bengal agar as described previously (5).

For seed treatment studies *Erfordia* seed containing 13% *A. fabae* was used in greenhouse and growth chamber (20°C) tests with four fungicides, benomyl (50%), captan (75%), thiram (75%), and carbathiin (75%) at rates of 4.2 g and 6.25 g of fungicide formulation per kg of seed. Four replicates each of 100 seeds were used in two greenhouse trials and in one growth chamber trial. Based on results from those tests, field trials were conducted using the 4.2 g rate only of benomyl, captan, and thiram. Four replicates of each treatment and an untreated control, each containing seven rows 17.5 cm apart and 5 m long, were sown in duplicate blocks in each of the years 1974 and 1975. In all experiments emergence counts were taken and some seedlings were removed from the soil and examined for symptoms of *A. fabae* infection 27-30 days after seeding. Sections from infected plants were plated on malt agar to verify the identity of the causal organism. Plants from the center row of the unsampled block were assessed for disease symptoms at 14-day intervals. At maturity the center row of each treatment in the unsampled block was harvested, weighed, and (100 X 4) seeds plated on malt agar in petri dishes to determine the amount of seed infection.

Results and discussion

Temperature

The optimum temperature for the growth of both *A. fabae* and *A. pisi* was 20°C on all media tested over the 14-day period. Radial growth was parallel on both faba bean agar (Fig. 1A) and pea agar (Fig. 1B), although *A. pisi* outgrew *A. fabae* slightly at all temperatures; however, colony diameters after 14 days were 5-15 mm less on faba bean agar than on pea agar. Radial growth of the two organisms on malt agar was similar at all temperatures for both organisms (Fig. 1C and D). It was found that *A. fabae* had the greatest radial growth after 14 days on malt agar, and hence malt agar was used as the culture medium throughout all subsequent experiments.

Infectivity

In preliminary tests it was found that disease symptoms were produced at temperatures of 15° , 20° and 25°C ,

with the optimum at 20°C . At 20°C well defined fruiting lesions were produced on leaves and stems 14 days after inoculation (Fig. 2 A, B). As there was little difference in efficiency among the three methods of inoculation in most tests, all subsequent tests used spore suspension sprayed directly onto unwounded leaves.

Cross-infection tests were negative; isolates of *A. pisi* from pea did not infect faba bean and *A. fabae* from faba bean did not infect pea. In England Beaumont (1) was successful in 2 of 25 attempts to inoculate *A. fabae* onto pea and in 2 of 13 attempts to inoculate *A. pisi* onto faba bean; he reported that sporulation occurred rarely but that typical *A. fabae* cultures were isolated from lesions on pea plants.

Survival in soil

A. fabae was not detected in any of 16 composite soil samples collected in the fall of 1974, after harvest or in the spring and fall of 1975. The inability of the fungus to survive even a few months in field soil is important, and indicates that in this area there is little danger of soil contamination from infected seed. Like *A. pisi*, *A. fabae* is probably distributed chiefly by infected seed.

Seed treatment

Although 13% of the seed used in these tests was infected, only 1-3% of the seedlings grown from that seed in the greenhouse or growth chambers became infected. This transmission rate was lower than the 4-8% transmission reported by Hewett (3). In one greenhouse trial, only 3 of 400 seedlings were infected in the control. One infected seedling was present in the carbathiin treatment and no infected seedlings were found in the captan, benomyl, or thiram treatments. In the second greenhouse trial, 1.75% of the seedlings were infected in the carbathiin and 0.5% in the thiram treatments, and no infected seedlings were found in the benomyl, captan, and control treatments. In growth chambers, the control contained 1.5% infected seedlings and all treatments had some infection but less than 0.5%.

In the 1974 field trial, initial infection levels in seedlings ranged from 0.4% with benomyl to 2.5% in the control (Fig. 3A). The highest plant infection level occurred in the captan treatment where 5% of the plants showed symptoms by August 1. By August 14 diseases plants were difficult to identify because of senescence accompanied by defoliation, and at harvest on September 16 no infected plants could be identified.

In the 1975 field trial (Fig. 3B), the initial infection level in seedlings was highest in the control at 1.7%. Once again infection levels reached a peak by the end of July (4-10%), although the plots had been seeded 21 days earlier than in 1974. Because of senescence and early defoliation, identifiable infection fell to zero on August 14. No significant differences occurred in the total yield or in 1000 kernel weight among treatments either year.

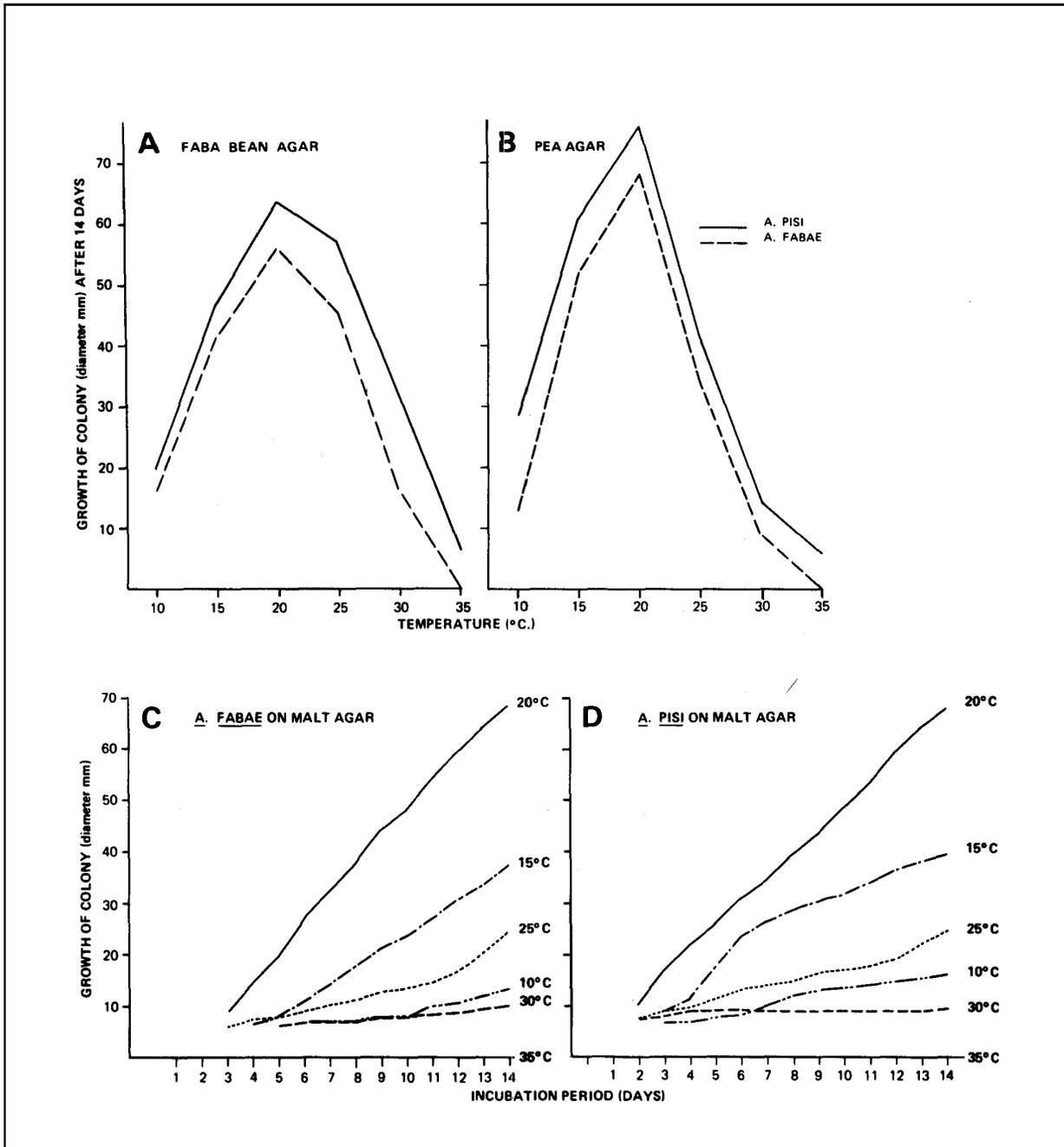


Figure 1. Radial growth of *Ascochyta fabae* and *A. pisi* colonies after 14 days at various temperatures on (A) faba bean agar and (B) pea agar; and effect of temperature on the radial growth of (C) *A. fabae* and (D) *A. pisi* on malt agar.

In 1974 *A. fabae* was isolated from 0.25% of the seed from the control and from 0.5% of the seed from the thiram-treated plots. No cultures of *A. fabae* were isolated from the seed of the captan- or benomyl-treated plots. In 1975, no cultures of *A. fabae* were isolated from the seed from any of the plots.

From these results it is apparent that for this disease to reach epiphytotic proportions, more favorable conditions would have to be present than occurred at Ottawa in 1974 and 1975. Despite an original seed-borne infection level of 13%, less than 3% of the seedlings became infected in the field, and the seed progeny from

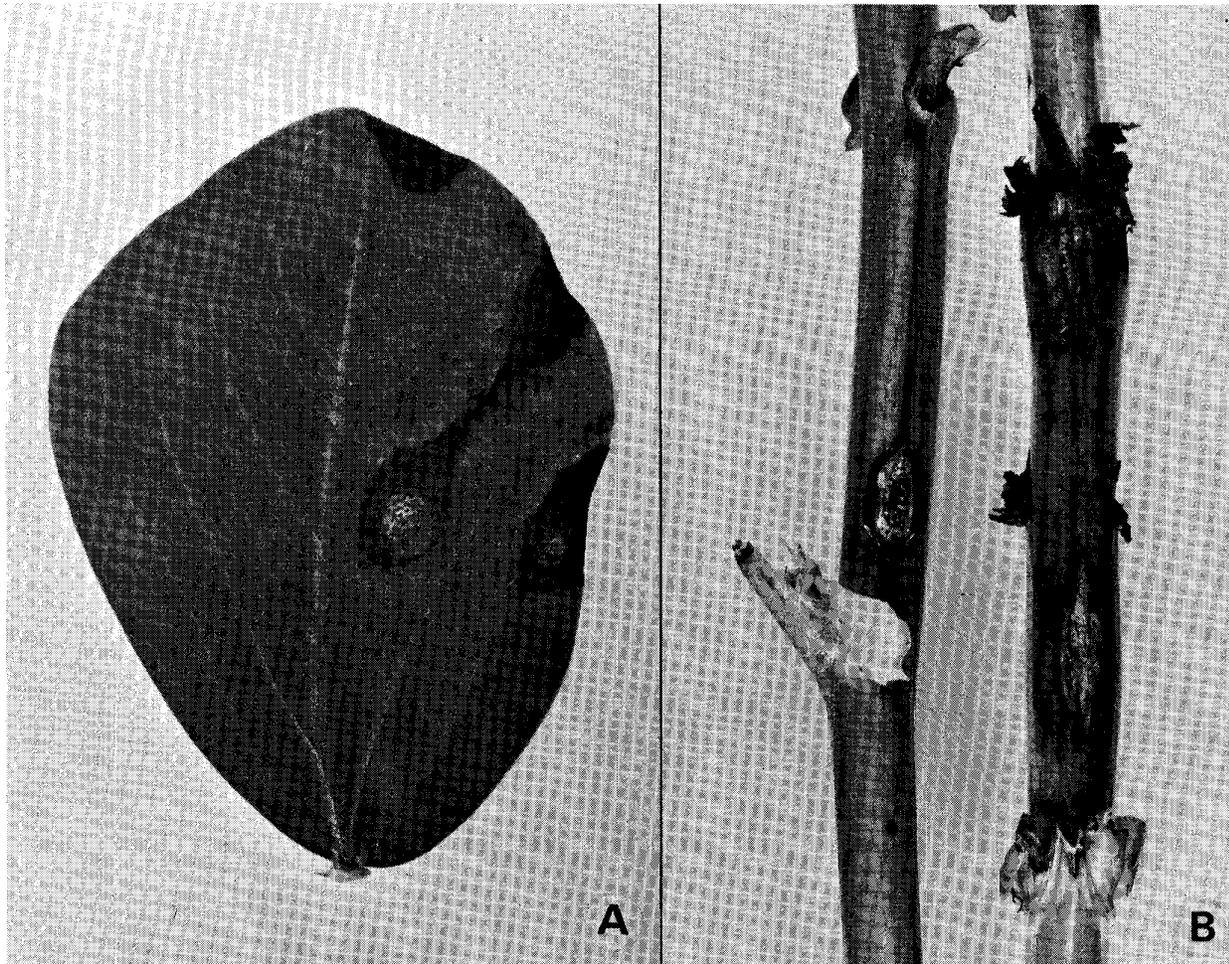


Figure 2. Lesions with pycnidia of *Ascochyta fabae* on inoculated *Erfordia faba* bean, A) leaf, B) stems.

None of the fungicide treatments gave complete control of seed borne infection.

Acknowledgment

The authors thank A.B. Ednie, Plant Products Division, Production and Marketing Branch, Agriculture Canada, for providing the seed samples of *Erfordia faba* bean.

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the 1974 and 1975 trials contained considerably less than 1% diseased seeds. Surveys conducted from 1972 to 1975 on faba bean seed grown at various locations across Canada showed that of 471 samples examined only 11 (2.3%) contained more than 5% infected seeds (unpublished results). This low rate of seed infection is not consistent with an aggressive disease pattern. However in England a severe outbreak of the disease coincided with an increased acreage of faba bean (3). There it was found that above-average rainfall in May and/or June was necessary for successful infection of the developing seedlings and it was usual for only 4-8% of the infected seeds in seed lots to produce diseased seedlings when sown in the field. The normal summer conditions in Ontario and the western provinces of Canada are not conducive to the buildup of this disease.

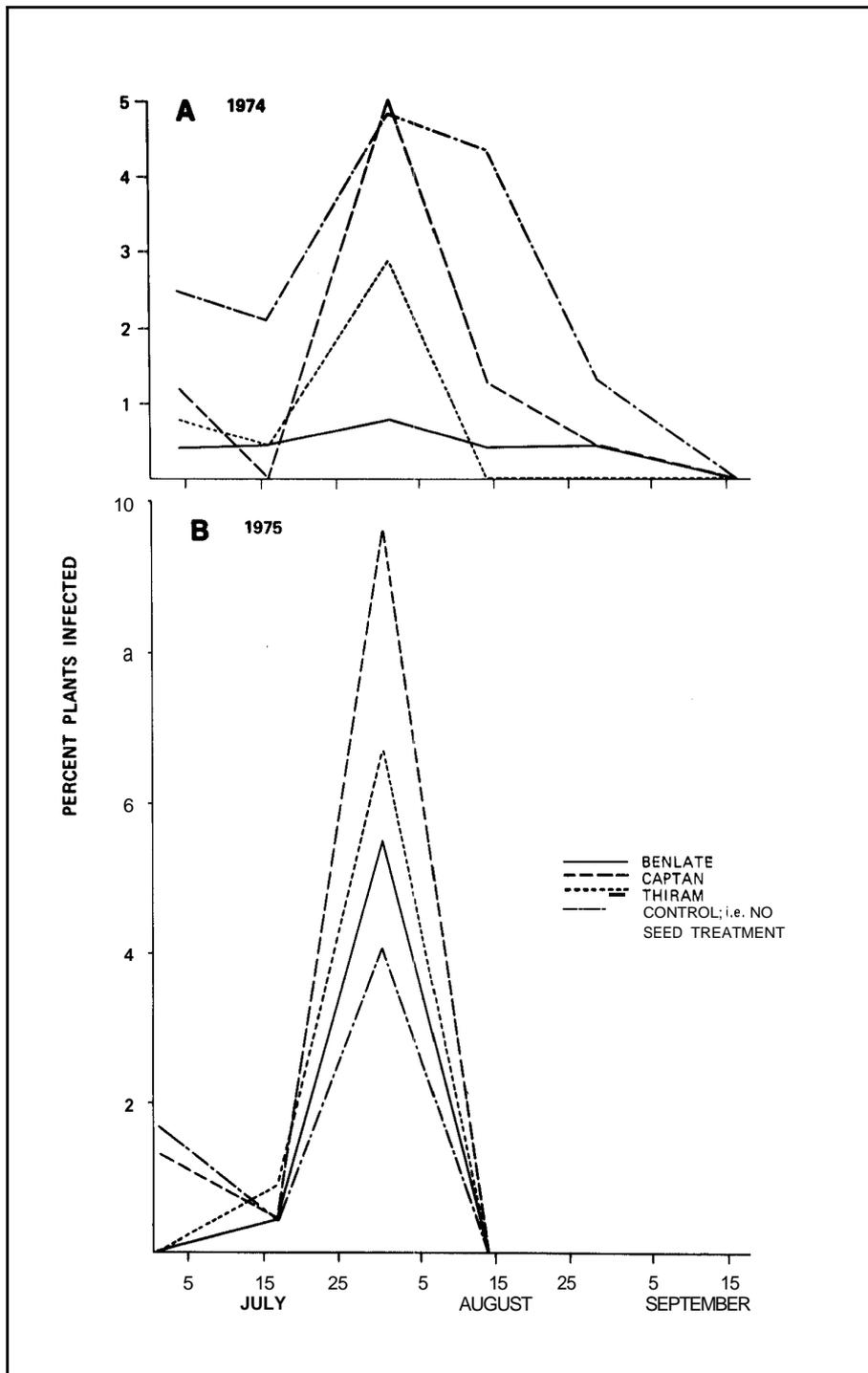


Figure 3. Effect of seed treatment on the control of seed-borne *A. fabae* and the progression of disease from uncontrolled seed-borne infection.

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