Determining Resistance Reactions of Field Pea Cultivars at the Seedling Stage to *Mycosphaerella pinodes*

R. C. Zimmer and Donald Sabourin

Research scientist, Agriculture Canada, P.O. Box 3001, Morden, Manitoba, Canada R0G 1J0; and biometrician, Agriculture Canada, Research Station, 195 Dafoe Road, Winnipeg, Manitoba, Canada R3T 2M9, respectively.
The authors thank I. Wolfe for technical assistance, and Mel Reimer for preparing the graphics.
Accepted for publication 30 January 1986.

ABSTRACT

Zimmer, R. C., and Sabourin, D. 1986. Determining resistance reactions of field pea cultivars at the seedling stage to *Mycosphaerella pinodes*. Phytopathology 76:878-881.

Four field pea (*Pisum sativum* var. *arvense*) cultivars were inoculated once, in the seedling stage, with several concentrations of pycnidiospores of *Mycosphaerella pinodes*. Parameters *u* and *r* of the logistic regression of log inoculum concentration on proportion leaf area infected were estimated for each cultivar and used to compare resistance across cultivars. Early in the incubation period, 4 days after inoculation, significant differences in the proportion of leaf tissue infected appeared among the four cultivars. With further incubation, 4–14 days after inoculation, the rates of disease progress among the cultivars varied but were not significantly different. Throughout the incubation period progress in development of the disease was greatest in cultivars Century and Trapper, followed, in order of increasing resistance, by Triumph and Tara. Resistance to development of this disease in field peas was influenced by the cultivar, seedling leaf age, and inoculum concentration. Under conditions marginally favorable for the pathogen, cultivars containing greater levels of rate-reducing resistance will become less severely diseased.

Additional key words: Ascochyta blight.

Ascochyta blight, a disease of field peas (*Pisum sativum* L. var. *arvense* (L. Poir.)) (5,7), is caused by *Ascochyta pinodes* (Jones) (teleomorph *Mycosphaerella pinodes* (Berk. and Blox.) Vestergr). It is probably the most destructive disease of field peas in Manitoba.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

Management of this disease by fungicide seed treatment, crop rotation, and sanitation is possible, but each has deficiencies. Resistance appears to be the more practical way to reduce its effect. Previous results indicate that the potential for finding a high-level source of resistance to this disease is low (1,5,7). However, by increasing the level of resistance in cultivars to buildup of this disease during the growing season, a partial degree of control may be obtained.

This paper presents a report on a study initiated to determine if differences in the proportion of leaf tissue infected could be

detected at the seedling stage among four field pea cultivars presently licensed in Canada.

MATERIALS AND METHODS

Inoculum. Inoculum was produced on an oatmeal-agar medium prepared by heating 100 g of rolled oats in 500 ml of distilled water at 70 C for 30 min. After heating, an additional 500 ml of distilled water was added, mixed well, and the resulting mixture filtered by vacuum, first through four, and then through eight layers of cheesecloth. Bacto agar was added to the filtrate to give a 1.8% agar concentration, and 10-ml aliquots of this medium were dispensed into test tubes, which were autoclaved and slanted. Several oatmeal-agar slants were flooded with a 1-ml conidial suspension from a virulent 7-day-old culture of *M. pinodes*. The inoculated slants were incubated at 20 C and subjected to a 16-hr photoperiod of fluorescent illumination at 5,000 lx.

The pycnidiospore inoculum was obtained from the slants by adding sterile distilled water to the 7-day-old cultures, rubbing the culture surface gently with a spatula, and placing the cultures on a shaker for 5 min to free the spores. The suspension was filtered through two layers of cheesecloth to remove large mycelial fragments. The pycnidiospore concentration of 500 per milliliter was determined with the aid of a hemacytometer and serial dilutions were made to produce pycnidiospore concentrations of 250, 125, and 75 per milliliter.

Inoculation and incubation. Several seed of field pea cultivars Century, Tara, Trapper, and Triumph were planted in a medium containing soil, sand, and peat moss (3:1:1, v/v). The seedlings, grown in a growth cabinet at 20–22 C, received 6,000–8,000 lx per 16-hr day. At the two-leaf stage the plants were thinned to three per 6-in. pot. At the six-leaf stage, approximately 19 days after planting, the plants in each pot were inoculated with a single spore concentration at 10 kPa (10 psi). To reduce inoculation variation the pots were rotated continuously as a 7-ml suspension of inoculum was directed at leaves three and four, counting from the base of the plant. After inoculation the plants were returned to the growth cabinet, in which two cool-mist type vaporizers were operated for 1 hr, shut off for 8 hr, operated for another hour, and following a 6-hr shut-off period, the vaporizers were removed and the cabinet turned on to resume normal operation. The inoculated plants received approximately 16 hr of moist incubation. The pots were arranged in a randomized complete block design with two pots per treatment per cultivar. Because of the small size of the growth cabinet this experiment was repeated five times.

Disease assessment. Disease assessments were made 4, 8, and 14 days after inoculation. Using the key for Stemphylium leaf spot of red clover (6) as a guide, the percentage of leaf area infected was estimated. The leaf area judged infected included, in addition to necrotic tissue, areas of the leaf that were pale green and had begun to dry and become brittle. In each replication of the experiment, the proportion of leaf area diseased was estimated on 12 leaflets for each treatment on each cultivar.

Statistical analyses. Apparent infection rates were not estimated in this paper because of the availability of only three assessments over time. Instead, a logistic regression equation (3), based on 96 data points, was estimated for each combination of assessment period, cultivar, and leaf position. The proportion of leaf area infected was hypothesized to be related to initial inoculum level by a logistic regression model of the form $(1 + e^{-r(D-u)-1})$, in which $D = \log^{10}$ inoculum concentration per milliliter and r and u are parameters to be estimated.

The proportion of leaf area infected is one-half when $u = D$. The parameter u can then be interpreted as the median infection parameter because it provides an estimate of the inoculum level required for any particular combination of cultivar, time, and leaf, for one-half of the leaf surface to be infected. An increase in u indicates an increase in initial resistance. Parameter r measures the slope of inoculum level on proportion of leaf area infected, and provides a measure of the progress of disease development for any combination of cultivar, assessment, and leaf. An increase in r indicates decreasing resistance over time.

Using this model, one can compare the resistance of cultivars, if assessment time and leaf number are controlled, by statistically comparing the values of r and u across cultivars. Cultivars whose 95% confidence intervals do not overlap are statistically different at the 5% level of significance. The parameters u and r were estimated separately for each cultivar and assessment period.

RESULTS

Initial reaction to infection. After 4 days of incubation, the initial reaction phase to infection, disease development was greatest in Trapper or Century, followed by Triumph and Tara in order of increasing resistance. Because this order was similar for both leaf ages, and the calculations were made over the entire

TABLE 1. Reaction of field pea cultivars to infection by inoculum of *Mycosphaerella pinodes* expressed by the estimated median infection parameter u (standard deviation in brackets) and 95% confidence interval for u in original inoculum concentration/ml scale[a]

Assessment[b] period	Cultivar	Median[c] infection u (sd)	95% confidence[c] interval for u in original scale
4	Trapper	2.18(.02)	(141-173)
	Century	2.28(.03)	(167-225)
	Triumph	2.40(.03)	(220-307)
	Tara	2.67(.04)	(415-596)
8	Century	1.67(.01)	(31-71)
	Trapper	1.87(.03)	(66-84)
	Triumph	2.17(.03)	(131-193)
	Tara	2.29(.04)	(181-259)
14	Century[d]	...	...
	Trapper[d]	...	...
	Triumph	1.67(.11)	(30-80)
	Tara	1.86(.04)	(61-91)

[a] Seedlings grown and after inoculation incubated in a growth cabinet operated at 20 C and a 16-hr photoperiod of 5,000 lx.
[b] Number of days after inoculation proportion of infected leaf area estimated.
[c] Median infection parameter u = an estimate of the log inoculum required for 50% of the leaf area to be infected; data is for all concentrations of inoculum combined.
[d] Data for Century and Trapper not included because 100% of the leaf area was diseased.

TABLE 2. Reaction of field pea cultivars to infection by inoculum of *Mycosphaerella pinodes* expressed by the estimated infection rate parameter r (standard deviation in brackets) and 95% confidence interval for r[a]

Assessment[b] period	Cultivar	Median[c] infection r (sd)	95% confidence[c] interval for r in original scale
4	Century	3.84(0.51)	2.84-4.84
	Trapper	7.49(1.19)	5.15-9.80
	Triumph	6.05(0.95)	4.19-7.91
	Tara	5.65(1.12)	3.45-7.86
8	Century	4.71(1.46)	1.84-7.57
	Trapper	6.86(1.48)	3.96-9.76
	Triumph	4.34(0.76)	2.84-5.84
	Tara	4.10(0.64)	2.84-5.36
12	Century[d]	...	...
	Trapper[d]	...	...
	Triumph	3.60(1.19)	1.27-5.94
	Tara	6.02(1.64)	2.81-9.23

[a] Seedlings grown and after inoculation incubated in a growth cabinet operated at 20 C and a 16-hr photoperiod of 5,000 lx.
[b] Number of days after inoculation proportion of infected leaf area estimated.
[c] Infection rate parameter r measures the rate at which infection progresses; data is for all concentrations of inoculum combined.
[d] The data for Century and Trapper not included because 100% of the leaf area was diseased.

inoculum concentration range, the results for leaves three and four were combined (Tables 1 and 2).

Disease development over time. The relationship of inoculum level to percent leaf area infected, for any cultivar-assessment period combination, appeared to follow a logistic distribution (Figs. 1 and 2). There was little evidence of significant differences among the cultivars in the rate of increase of infected leaf tissue over time (Table 2); nevertheless, the rate of disease development appeared to differ among cultivars (Figs. 1 and 2). The disease in general progressed more quickly in Century and Trapper than in Tara and Triumph.

In leaf three, the older of the two leaves inoculated, the disease developed more quickly on Trapper than on Century early in the incubation period (4 days post-inoculation); this occurred at all inoculum concentrations. Also, at all inoculum concentrations, the proportion of leaf tissue infected increased more quickly in Triumph than in Tara. After 14 days incubation 100% of the leaf area was infected in Century at all inoculum concentrations, whereas in Trapper 90–100% was diseased. In Triumph and Tara 90–100% was diseased at 125, 250, and 500 spores per milliliter; at 75 spores per milliliter 80 and 70%, respectively, was diseased in these cultivars.

In leaf four, the younger leaf, at inoculum concentrations of 250 and 500 spores per milliliter, the disease had developed more quickly, 4 days after inoculation, in Trapper than in Century; at 75 and 125 spores per milliliter Century was slightly more severely diseased. After 14 days incubation, over 90% of the leaf area was infected at all inoculum concentrations in Century and Trapper and at the 500 spores per milliliter concentration in Tara. In Triumph and Tara and in Triumph at 250 and 500 spores per milliliter, respectively, 85–90% of the leaf area was infected. At 125 spores per milliliter the proportion of leaf area infected in Triumph and Tara was 72 and 62%, respectively; at 75 spores per milliliter 60 and 35%, respectively, of the leaf area was infected.

If progress of disease development at the various inoculum concentrations is compared on leaves three and four, 8 days after inoculation, the proportion of infected leaf tissue was greater on leaf three than on leaf four (Figs. 1 and 2). At 75 spores per milliliter the proportion (%) of infected leaf area for leaves three and four on Century, Trapper, Triumph, and Tara, respectively, was: 78/57, 54/51, 26/16, and 31/5%; for 125 spores per milliliter: 86/78, 81/68, 48/31, and 35/10%; for 250 spores per milliliter: 94/94, 96/96, 77/65, and 62/39%; and for 500 spores per milliliter: 100/97, 100/99, 96/72, and 93/62%.

DISCUSSION

Because of the intractable nature of Mycosphaerella blight, control of this disease is difficult. Resistance is the most efficient means of control, but previous research results indicate that the probability of finding a strong source of resistance is low (1,5,7).

The results reported in this paper indicate that the rate of development of this disease was affected by the germ plasm of the field pea cultivars tested. Early in the incubation period the rate of disease development varied significantly among the cultivars; with subsequent incubation those differences, although not significant, remained apparent. The disease developed more quickly in Century and Trapper than in Tara and Triumph, and the pattern of development of the disease in Century and Trapper was similar. The high rates and high similarity in pattern of disease development between Century and Trapper may have been due to the similarity of their parentage.

The differences among cultivars in the rate of disease progress were also affected by inoculum concentration and leaf age. As inoculum concentration increased the rate of disease development over time increased rapidly, then decreased. The effect of leaf age was observed throughout the incubation period at the lower inoculum concentrations or early in the incubation period at the

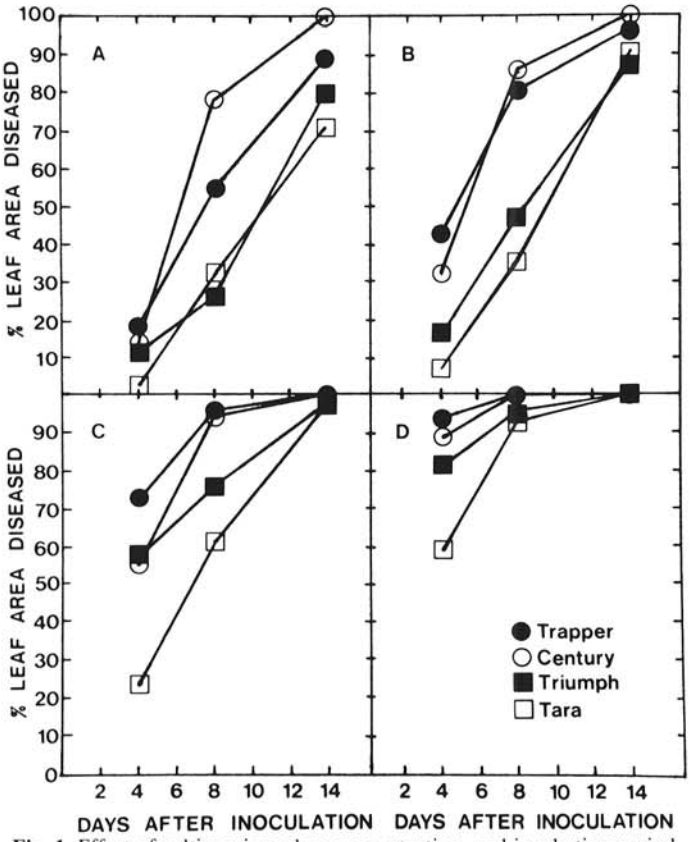

Fig. 1. Effect of cultivar, inoculum concentration, and incubation period after inoculation on percentage of leaf area infected on leaf three in four field pea cultivars inoculated with pycnidiospore suspensions of *Mycosphaerella pinodes*. **A,** 75 spores per milliliter; **B,** 125 spores per milliliter; **C,** 250 spores per milliliter; **D,** 500 spores per milliliter.

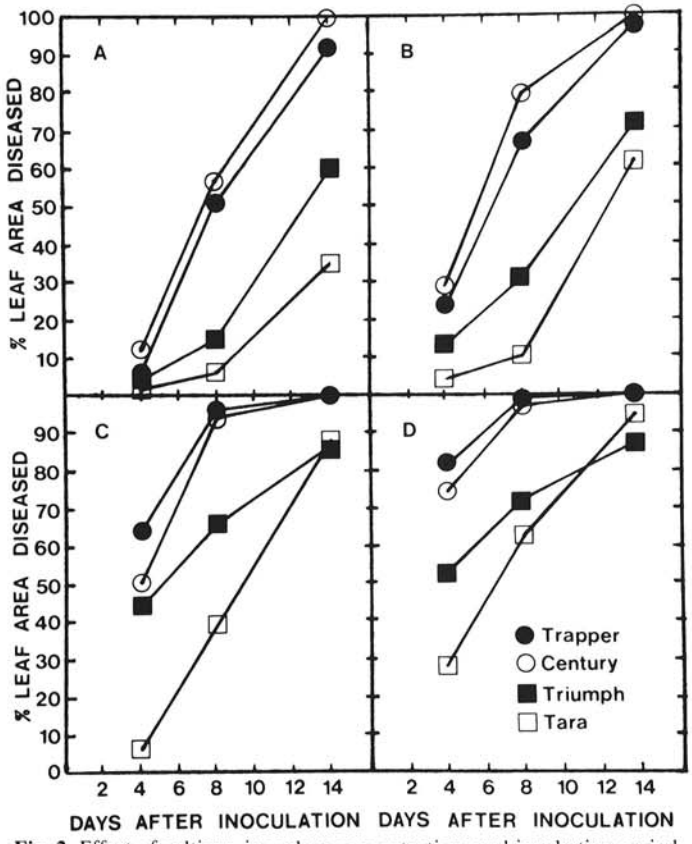

Fig. 2. Effect of cultivar, inoculum concentration, and incubation period after inoculation on percentage of leaf area infected on leaf four in four field pea cultivars inoculated with pycnidiospore suspensions of *Mycosphaerella pinodes*. **A,** 75 spores per milliliter; **B,** 125 spores per milliliter; **C,** 250 spores per milliliter; **D,** 500 spores per milliliter.

higher inoculum concentrations. The rate of increase was greater in the older leaf than in the younger leaf. It may be that a substance inhibitory to the pathogen, such as the phytoalexin pisatin, was formed as result of infection by the pathogen. If such was the case, it follows that lesser quantities of the inhibitory substance were formed in the more susceptible cultivars and in the older leaf. Bailey (2) found in leaf disks and detached leaves that pisatin production decreased as the leaves senesced. Although the leaves on these plants were not senescing, they may have possessed different potentials to produce such a substance.

Cultivars that possess rate-reducing resistance will suppress development of disease under conditions marginally favorable for the pathogen (4). Under such environmental conditions Tara and Triumph will be less severely diseased than Century and Trapper. However, if the environment is favorable and large populations of the pathogen are present, the disease is likely to become severe on all four cultivars.

Until a source of strong resistance is obtained to the Mycosphaerella blight pathogen, detection and incorporation of a high level of rate-reducing resistance into commercial cultivars offers the best approach to reducing the effects of this disease. It has been shown, under natural conditions, that this disease progresses up plants of Triumph and Tara more slowly than on plants of Century and Trapper (R. Zimmer, *unpublished*).

LITERATURE CITED

1. Ali-Khan, S. T., Zimmer, R. C., and Kenaschuk, E. O. 1973. Reaction of pea introductions to Ascochyta foot rot and powdery mildew. Can. Plant Dis. Surv. 53:155-156.
2. Bailey, J. A. 1969. Phytoalexin production by leaves of *Pisum sativum* in relation to senescence. Ann. Appl. Biol. 64:315-324.
3. Cox, D. R. 1966. Some procedures connected with the logistic response curve. Pages 55-71 in: Research Papers in Statistics: Essays in honor of Neymans 70th birthday. F. N. David, ed. John Wiley & Sons, London and New York.
4. Fry, W. E. 1982. Principles of plant disease management. Academic Press, New York. 378 pp.
5. Hare, W. W., and Walker, J. C. 1944. Ascochyta diseases of canning pea. Wisc. Agric. Exp. Stn. Res. Bull. 150. 31 pp.
6. James, C. 1971. A manual of assessment keys for plant diseases. Canada Dept. Agric. Publ. 1458.
7. Jones, L. K. 1927. Studies of the nature and control of blight, leaf and pod spot and foot rot of peas caused by species of Ascochyta. NY. State Agric. Exp. Stn. Bull. 547. 46 pp.