Quantitative Inoculations with *Erysiphe pisi* to Assess Variation of Infection Efficiency on Peas

P. Reeser, D. J. Hagedorn, and D. I. Rouse

Research assistant, professor, and assistant professor, respectively, Department of Plant Pathology, University of Wisconsin, Madison 53706.

Supported by Project 120, University of Wisconsin, College of Agricultural and Life Sciences.
Accepted for publication 15 February 1983.

ABSTRACT

Reeser, P., Hagedorn, D. J., and Rouse, D. I. 1983. Quantitative inoculations with *Erysiphe pisi* to assess variation of infection efficiency on peas. Phytopathology 73:1238-1240.

Two techniques were developed for quantitative inoculation of pea (*Pisum sativum*) with the powdery mildew fungus, *Erysiphe pisi*. In the first, a liquid carrier composed of 0.0025% Tween-20 in 0.1% water agar was used. In the second, dry Sephadex G-25 (fine grade) was the inoculum carrier. The liquid inoculum was used in tests designed to differentiate resistant and susceptible pea isolines and to identify the stage in the infection process where resistance was expressed. The dry Sephadex inoculum carrier was also used successfully, although variability within replicates was higher than for the liquid carrier.

Recently it has been suggested that real advantages accrue from using rate-reducing (horizontal) resistance in the control of plant disease (14). However, screening for horizontal resistance has been hindered by a dearth of rapid and reliable methods of measurement. Reliability is a particularly significant problem. By definition, rate-reducing resistance must be quantitative; however, for various plant diseases, including the powdery mildews, screening for the components of this type of resistance has been burdened by the lack of quantitative inoculation techniques.

In resistance evaluations excised leaves or leaf disks are often inoculated with powdery mildew conidia in a settling tower (8,9,11). Usually these conidia are introduced into the settling tower by shaking diseased plants or leaves over the tower. Milligram quantities of conidia of *Erysiphe graminis* DC f. sp. *hordei* Marchal have been collected and subsequently discharged directly into a settling tower with an apparatus specifically designed for that purpose (2). With either of these techniques, it is difficult to control inoculum density and uniformity. Camel's-hair brushes may be used to transfer conidia from sporulating colonies to leaves or leaf disks by gentle stroking or tapping; however, this method is time consuming and is also difficult to quantify (10,12). Another technique for applying dry inoculum utilizes a cotton swab for transferring conidia (13).

The literature pertaining to the effects of relative humidity and free water on powdery mildew conidia is discussed in several reviews (4,19,21). Although it is generally acknowledged that free water reduces the viability of these conidia, aqueous suspensions containing powdery mildew conidia have been used for inoculating host tissues (1,6,15,16). There are few data on the suitability of aqueous suspensions in quantitative methods. A nonaqueous liquid, perfluorotributylamine, has been used to suspend conidia of *E. graminis* f. sp. *hordei*, but inoculations performed with that carrier did not produce more colonies than dusting the leaves with dry conidia (3).

Reeser and Hagedorn (16) demonstrated for the first time the possibility of using dry powder inoculum carriers for *E. pisi* DC for studies of pea powdery mildew.

This paper describes an investigation of two techniques for quantitatively applying conidia of this pathogen to leaves of pea (*Pisum sativum* L.).

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.

MATERIALS AND METHODS

E. pisi was initially obtained from naturally infected plants in a greenhouse at the University of Wisconsin in Madison. The fungus was maintained on young pea plants (cultivar Dark Skin Perfection) grown in 15-cm-diameter pots in greenhouse potting soil. Plants were maintained in the greenhouse at 20–24 C with 16 hr daily of supplemental fluorescent light. Light intensity was about 105 $\mu E \cdot s^{-1} \cdot cm^{-2}$ at 400 nm. Fresh colonies were started each week by shaking the foliage of infected plants over adjacent younger plants.

Powdery mildew-free plants were grown in 10-cm-diameter clay pots in potting soil (four plants per pot) in an isolated growth chamber at 20–24 C under fluorescent and incandescent lights (200 $nEs^{-1}cm^{-2}$) with a 12-hr photoperiod. The upper two mature, fully expanded leaves were excised from these plants when they were 30–35 days old by making an abaxially beveled cut through the petiole near the stem with a sterile razor blade. Excised leaves were maintained in leaf culture chambers prepared by stretching two layers of Parafilm "M," one at a time, over the bottom section of a glass petri dish, and filling the dish with sterile double distilled water. The nondisinfested petioles of excised leaves were inserted through holes punched in the Parafilm and into the water. A 7-cm-tall flexible plastic sleeve was fitted around the dish, and the lid was placed on this sleeve to prevent contamination prior to inoculation. After the leaves were inoculated, they were incubated with the chamber lids removed at ambient laboratory room temperature under two 40-W Gro-Lux fluorescent tubes placed approximately 30 cm above the leaf surface. Room relative humidity was 40–70%, but within the chambers at leaf surface it was likely $\geq$90%. Results of preliminary investigations showed that the addition of dilute Hoagland's solution, sucrose, kinetin, or benzimidazole to the distilled water at various concentrations did not delay leaf senescence. Benzimidazole at 10 or 100 ppm actually reduced leaf longevity and inhibited infection by *E. pisi*.

Two experiments were performed to test the feasibility of using either dry or liquid inoculum carriers to quantitatively study infection efficiency as a component of resistance to *E. pisi*. In these (and subsequent) experiments the most acceptable dry and liquid inoculum carriers as determined in a series of similar preliminary experiments were used. In the preliminary experiments, variability between inoculations was not assessed and it was possible to determine only whether colonies were or were not formed following application of the conidia in the carriers that were tested. No direct comparison could be made between different "successful" carriers.

In experiment I, inoculum was prepared by vacuuming conidia from the stock cultures into a small plastic tube with a cyclone

spore collector, adding 1 g of Sephadex G-25 fine grade and mixing this thoroughly on a Vortex mixer. This inoculum was dispensed into an octagonal cardboard settling tower (35 cm diameter × 100 cm tall) from a 250-ml bottle, through a DeVilbiss model 119 insufflator attached to a Schein inoculator (18) to control air pressure at 4.2×10^4 N/m^2 during the 5 sec application time. Inoculum was directed upward from a nozzle in the base of the settling tower and was allowed to settle on the leaves for 5 min.

Excised leaves of the cultivars Little Marvel, Dark Skin Perfection, 8221, New Era, and New Season were inoculated according to a randomized complete block design. Plants of each of the five cultivars were placed in randomized positions in the settling tower, for each of five serial inoculations (replications). Time between serial inoculations was less than 10 min. Between each such test plant inoculation, 2% water agar check plates were alternately inoculated to monitor inoculum density and viability over the course of the experiment. The conidial germination rate was estimated by counting germinated conidia on the agar surface with the aid of a microscope (×125, bright field) along a transect (1.70 × 50 mm) radial to the center (spray nozzle) of the settling tower.

Inoculated leaves were incubated for 5–7 days, after which the number of colonies per leaf were counted with the aid of a dissecting microscope (×20). Leaf areas were determined with a Li-cor model 3100 area meter (Lambda Instruments Corp., Lincoln, NE 68504) and the number of colonies per square centimeter of leaf surface was computed.

In experiment II, inoculum was prepared by vacuuming conidia from the stock cultures into an autoclaved solution of 0.1% water agar and 0.0025% Tween-20. Conidia were held for periods of up to 4 hr in suspension with no apparent effect on viability. This inoculum was atomized upward through a glass chromatography sprayer fixed in the base of the settling tower as described above. A suspension containing 8×10^4 conidia per milliliter applied with the Schein inoculator calibrated to 4.2×10^4 N/m^2 and duration of spray at 5 sec delivered approximately 50 conidia per square centimeter. Liquid inoculum carrier was used in studies of pea plants grown from seeds of the susceptible (Sprite, Dark Skin Perfection, New Era, and New Line Early Perfection) and their respective resistant near-isogenic lines (Wisconsin 7101, 7102, 7103, and 7104) provided by E. T. Gritton (7).

Excised leaf culture chambers were placed in the settling tower so that leaves of four plants from each of a given pair of near-isogenic lines were inoculated at one time. After 3–5 days of incubation, leaves were sampled in the following manner (5): double-stick cellophane tape was placed on glass microscope slides which were then firmly pressed against the leaf surface. After the leaf was peeled off, a drop of lactophenol and 0.5% cotton blue was placed on each replica and covered with a 22-mm square coverslip. The stained leaf surface replicas were examined at ×125. Five transects were taken across each inoculation replication, and the data were recorded as the number of ungerminated conidia, the number of conidia with germ tubes, the number of conidia that formed appressoria but grew no further, and the number of colonies. These data were used to compute inoculum density as conidia per square centimeter of leaf surface, proportion of conidia germinated, the proportion of germinated conidia that matured past the germ tube stage, the proportion of appressorial conidia that matured to produce elongating secondary hyphae or colonies, and the infection efficiency (proportion of conidia deposited that formed colonies).

RESULTS

In preliminary experiments, several types of dry powders were tested as inoculum carriers. Dry cornmeal and powdered egg albumin inoculum carriers both gave uniform dispersal of the inoculum, but they also served as substrates for growth of saprophytic fungi. Medium and fine grade Sephadex G-25 did not support growth of saprophytic fungi when used as inoculum carriers. The two grades of Sephadex did not differ significantly in their effect on inoculum density or infectivity. However, although the viability of conidia in the dry inoculum carrier was fairly high (32–39% germination on 2% water agar), infection efficiencies were fairly low (only 3.7% of conidia deposited produced colonies).

Results presented in Table 1 indicate the effectiveness of the dry inoculum. Mean inoculum densities were uniform across individual leaves and plate positions within each serial inoculation, whereas differences across serial inoculations were small but significant. Densities on each plate ranged from 91–150 conidia per square centimeter (25 plates observed) with an overall mean of 119.4 conidia per square centimeter (s.e. = 11.4). Differences in inoculum viability expressed as mean proportion of conidia germinated were also small, but viability was significantly reduced in the latter inoculations.

Mean number of colonies per square centimeter formed on leaves of cultivars Little Marvel, New Era, Dark Skin Perfection, 8221, and New Season were 6.8, 4.7, 3.7, 3.5, and 3.3, respectively. The number of colonies formed differed significantly across serial inoculations, but results were similar to those obtained from the alternate serial inoculations of water agar plates. The mean number of colonies per square centimeter of leaf surface was significantly higher in plants of Little Marvel, while counts for cultivars New Season and 8221 showed significantly ($P = 0.05$) lower rates of colony formation than those of cultivars Little Marvel and New Era. This confirmed results obtained in a previous experiment.

The inoculum carrier composed of 0.1% water agar and 0.0025% Tween-20 gave consistently good results when used for inoculating excised pea leaves with conidia of *E. pisi*. In a series of preliminary experiments conducted at temperatures between 16 and 24 C, the proportion of conidia germinated on the leaf surface using either the 0.1% water agar or the 0.0025% Tween-20 inoculum carrier ranged between 0.62 and 0.77 on susceptible cultivars. Infection efficiency (proportion of conidia that formed colonies) ranged from 0.17 to 0.44 depending on incubation temperature and cultivar. Furthermore, this inoculum carrier was found to have little effect on conidial viability compared to those of various other surfactant solutions (Table 2).

Excellent uniformity in inoculum density was obtained with the carrier composed of 0.1% water agar plus 0.0025% Tween-20. Eight sequential inoculations resulted in mean inoculum densities (conidia per square centimeter) of 24.4, 27.0, 27.7, 33.1, 30.2, 27.8, 48.5, and 24.8, respectively. Only one large deviation from the mean (inoculation 7 = 48.5) occurred; this was attributed to a malfunction of the Schein inoculator.

Germination percentages were closely similar for the susceptible pea cultivars Sprite, Dark Skin Perfection, New Era, and New Line Early Perfection. However, dramatic differences in infection efficiency were obtained when they were compared with their resistant isolines by using a liquid inoculum carrier containing 0.1% water agar and 0.0025% Tween-20. No infection occurred on plants of the isolines; infection efficiency data for the above cultivars were 0.27, 0.29, 0.38, and 0.44, respectively.

Careful, repeated study of the infection efficiency phenomenon showed, for the first time, why the leaves of certain pea cultivars are

TABLE 1. Effect[y] of serial inoculation with dry inoculum on density and viability of *Erysiphe pisi* conidia on 2% water agar check plates

Serial inoculation	Mean inoculum density (conidia/cm^2)	Mean proportion of conidia germinated[z]
1	126.8 a	0.37 a
2	103.2 c	0.36 a
3	112.2 bc	0.30 b
4	122.4 ab	0.30 b
5	132.4 a	0.28 b

[y] Based on a two-way ANOVA (Minitab II) for serial inoculation ($P = 0.02$) and plate position in the settling tower ($P = 0.05$). Means followed by the same letter are not significantly different as determined by Duncan's multiple range test ($P = 0.05$).

[z] Based on a one-way ANOVA (Minitab II) for serial inoculations ($P = 0.0025$). Means followed by the same letter are not significantly different as determined by Duncan's multiple range test ($P = 0.05$).

TABLE 2. Effect of various surfactants in the inoculum carrier on the mean proportion of *Erysiphe pisi* conidia germinated on 2% water agar

Surfactant	Concentration (% v/v)	Mean proportion of conidia germinated[x]
Tween-80	0.001	0.89 a[y]
Tween-20	0.001	0.86 ab
Tween-80	0.01	0.86 ab
Tween-20	0.01	0.83 ab
Tween-20	0.1	0.82 ab
Tween-80	0.1	0.79 bc
Control[z]		0.79 bc
Triton X-100	0.001	0.73 c
Nonanol	0.0001	0.65 d
Nonanol	0.01	0.63 de
Tergitol NPX	0.001	0.57 ef
Nonanol	0.001	0.55 f
Triton X-100	0.01	0.27 g
Tergitol NPX	0.01	0.25 g
Tergitol NPX	0.1	0.02 h
Triton X-100	0.1	0.01 h

[x]Means represent the average of five observations on each of three replications for each treatment.
[y]Means followed by the same letter are not significantly different as determined by Duncan's multiple range test, $P = 0.05$.
[z]Conidia shaken from infected leaves directly onto water agar.

resistant to *E. pisi*. Conidia generally germinate and appressoria are formed but these do not successfully give rise to an established infection.

DISCUSSION

The inoculum carrier composed of 0.1% water agar and 0.0025% Tween-20 permitted more accurate adjustment of inoculum density than the dry carrier composed of Sephadex G-25 (fine grade). Viability of conidia in the liquid carrier was approximately twice that in the dry carrier, and infection efficiencies were nearly 10-fold greater when conidia were suspended in the liquid carrier.

The liquid inoculum carrier represents a distinct improvement over previous methods for inoculating plants with conidia of species of the Erysiphaceae. It is inexpensive to prepare and easy to use. When combined with a quantitative inoculator and a settling tower, the liquid carrier allows a high degree of control over inoculum density and uniformity, both within and between experiments, reducing effects due to variability in these factors. This should help increase the precision of experiments designed to measure rate-reducing resistance, to study mechanisms of resistance, and to study genetics of resistance and pathogenicity.

Intermediate levels of resistance that reduce the rate of disease progress of powdery mildew of peas in commercially grown cultivars has been observed in the field (15). The development of precise methods of inoculation reported allowed comparisons to be made between cultivars for one possible component of rate-reducing resistance. The results indicated significant differences in infection efficiency of *E. pisi* on plants of commercial pea cultivars. Infection efficiency may be an important component of rate-reducing resistance. It has been shown theoretically for the wheat powdery mildew system that when infection efficiency is low, a small negative change in this character may yield a significant increase in rate-reducing resistance (17). Shaner (20) demonstrated that slow mildewing of Knox wheat was due both to reduced infection efficiency and to reduced sporulation capacity. We did not compare the pea cultivars used in this study for components of rate-reducing resistance other than infection efficiency. Neither was an attempt made to compare these pea cultivars under similar field conditions. Thus, the degree of effectiveness of a given reduction in infection efficiency to reduce disease progression in the field is not known for the cultivars used.

The inoculation technique may be useful in a breeding program for rate-reducing resistance by providing a screening method allowing selection on the basis of macroscopic evaluation of colony density on an individual plant basis.

LITERATURE CITED

1. Aldwinckle, H. S., Watson, J. P., and Gustafson, H. L. 1975. Relationship between greenhouse and field resistance of grape seedlings to powdery mildew. Plant Dis. Rep. 59:185-188.
2. Aust, H. J., and Kranz, J. 1974. Einflüss der Konidiendichte auf die Keimung, Infektio, Inkubationszeit und Sporulation bei dem echten Mehltan der Gerste (*Erysiphe graminis* DC f. sp. *hordei* Marchal). Phytopathol. Z. 80:41-53.
3. Bushnell, W. R., and Rowell, J. B. 1967. Fluorochemical liquid as a carrier for spores of *Erysiphe graminis* and *Puccinia graminis*. Plant Dis. Rep. 51:447-448.
4. Butt, D. J. 1978. Epidemiology of powdery mildews. Page 61 in: The Powdery Mildews. D. M. Spencer, ed. Academic Press, London.
5. Delp, C. J. 1954. Effect of temperature and humidity on the grape powdery mildew fungus. Phytopathology 44:615-626.
6. Ford, R. E., and Baggett, J. R. 1965. Reactions of plant introduction lines of *Pisum sativum* to alfalfa mosaic, clover yellow mosaic, and pea streak viruses, and to powdery mildew. Plant Dis. Rep. 49:787-789.
7. Gritton, E. T., and Hagedorn, D. J. 1971. Registration of Wisconsin pea cultivars. Crop Sci. 11:941.
8. Jhooty, J. S., and McKeen, W. E. 1965. Studies on powdery mildew of strawberry caused by *Sphaerotheca macularis*. Phytopathology 55:281-285.
9. Jones, I. T. 1975. The pre-conditioning effect of day-length and light intensity on adult plant resistance to powdery mildew in oats. Ann. Appl. Biol. 80:301-309.
10. Lim, T. M. 1973. A rapid laboratory method of assessing susceptibility of *Hevea* clones to *Oidium heveae*. Exp. Agric. 9:275-279.
11. Mignucci, J. S., Lim, S. M., and Hepperly, P. R. 1977. Effects of temperature on reactions of soybean seedlings to powdery mildew. Plant Dis. Rep. 61:122-124.
12. Mukhopadhyay, A. N., and Russell, G. E. 1979. Development of *Erysiphe betae* on leaves of four sugar beet varieties. Phytopathol. Z. 96:15-20.
13. Nair, K. R. S., and Ellingboe, A. H. 1962. Method of controlled inoculations with conidiospores of *Erysiphe graminis* var. *tritici*. Phytopathology 52:714.
14. Nelson, R. R. 1978. Genetics of horizontal resistance to plant diseases. Annu. Rev. Phytopathol. 16:359-378.
15. Raut, B. T., and Wangikar, P. D. 1979. Varietal reaction of peas to the powdery mildew. Indian Phytopathol. 32:282.
16. Reeser, P. W., and Hagedorn, D. J. 1981. A method for quantitative inoculation with powdery mildew (*Erysiphe pisi*) of pea. Phytopathology 71:901.
17. Rouse, D. I. 1979. Components of horizontal resistance to powdery mildew of wheat as related to disease progress and parasitic fitness. Ph.D. thesis, The Pennsylvania State University, State College, PA. 92 pp.
18. Schein, R. D. 1964. Design, performance, and use of a quantitative inoculator. Phytopathology 54:509-513.
19. Schnathorst, W. C. 1965. Environmental relationships in the powdery mildews. Annu. Rev. Phytopathol. 3:343-366.
20. Shaner, G. 1973. Reduced infectibility and inoculum production as factors of slow mildewing in Knox wheat. Phytopathology 63:1307-1311.
21. Yarwood, C. E. 1957. Powdery mildews. Bot. Rev. 23:235-301.