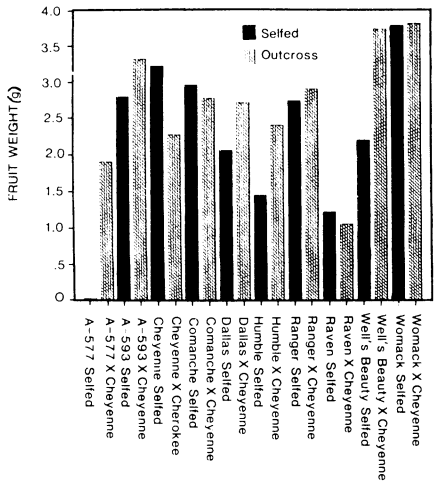

Fig. 2. Effect of cross- and self-pollination on mean fruit weight of blackberry cultivars.

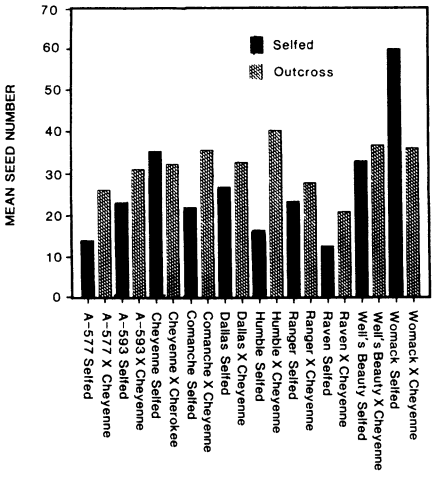

Fig. 3. Effect of cross- and self-pollination on seed number in fruits of blackberry cultivars.

in pollination; and 3) field grown plants exposed to both wind and insect pollination. Four replications of 'Cheyenne' were used for each treatment, and 40 fruits from each replication were weighed, and seed numbers were determined.

No significant differences in percentage fruit set from cross or self pollination occured in the named cultivars (Fig. 1). Only the selections, A-577 and A-593, showed a decreased set in self-pollinated flowers. A-577, resulting from a cross of 'Whitford Thornless' x 'Early Harvest', was the only diploid clone in the test. Although A-593 is tetraploid, it originated from the cross 'Brazos' (4x) x 'Hillquist' (2x) and apparently resulted from an unreduced gamete of 'Hillquist'. Thus, the only clones in the study that exhibited self-incompatibility were from diploid ancestry. Most of the cultivars responded similarly in percentage fruit set following cross-pollination, although 'Wells Beauty' showed somewhat less fruit set than the other cultivars.

Although pollen source did not affect percentage fruit set in most cultivars, self-pollination reduced average fruit weight in several cultivars (Fig. 2), most notably A-577, 'Dallas', 'Humble', and 'Wells Beauty'. Little or no reduction occurred in other cultivars. 'Cheyenne' produced significantly smaller fruit when pollinated by 'Cherokee' than when self-pollinated, but this effect was not apparent in fruit set. Lawrence (5) noted that pollen source in blackberry combinations appears to be important in drupelet set.

Self-pollination resulted in reduced seed number in fruits of most cultivars (Fig. 3). Notable exceptions were 'Cheyenne' and 'Womack', in which seed number in self-pollinated fruits was as great or greater than in cross-pollinated fruits.

Microscopic observation of pollen tube growth through the styles of blackberry flowers using fluorescence techniques showed no difference in rate or extent of growth between cross or self-pollens. Pollen tubes from both pollen sources grew the full length of the styles and into the ovary region during the 48 hr incubation period.

A comparison of fruit size and seed number from open pollinations in a greenhouse, open field, and insect-excluded field cages showed that blackberry flowers are not dependent on insects for pollination (Table 1). Caged plants produced larger fruits with more seeds, than uncaged plants, but this may have been due to lower temperatures from shading whereas the uncaged field plots were exposed to unseasonably high temperatures in 1980. The small fruits with few seeds produced by greenhouse grown plants likely resulted from inadequate pollen dispersal within the flower. Fruits produced on these plants were malformed, indicating a lack of pollination of all pistils in the aggregate flower.

Table 1. Effect of method of pollination on fruit size and seed number in 'Cheyenne' blackberry.

Location of plants	Fruit size (g)	Seeds per fruit (no.)
Field-caged	4.73 a[z]	59.2 a[z]
Field-open	3.86 b	45.9 b
Greenhouse	2.95 c	27.7 c

[z]Mean separation in columns by Duncan's multiple range test, 5% level.

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Inoculation Methods for Evaluating Verticillium Wilt Resistance in Strawberry Germplasm

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Abstract. **Four methods of inoculation with *Verticillium* were tested for effectiveness in infecting strawberry plants grown in a greenhouse bench. The most severe and early symptoms were produced with a macerated mycelium root dip inoculum. Effect of inoculum aggressiveness on the extrapolation of plant resistance information is discussed.**

The USDA strawberry (*Fragaria* ×*ananassa* Duch.) breeding program has emphasized for many years *Verticillium* wilt resistance as a criterion for cultivar development. In the past, we used field screening tests to determine levels of *Verticillium* wilt resistance in advanced selections. However, in the past 5 years we have developed a satisfactory method to screen plants in a greenhouse bench test in which plant and pathogen interactions can be controlled better than in field plots (2). Concerns with the greenhouse bench test have included the suitability of the type of inoculum used, its method of application to the plants to be tested, and the var-

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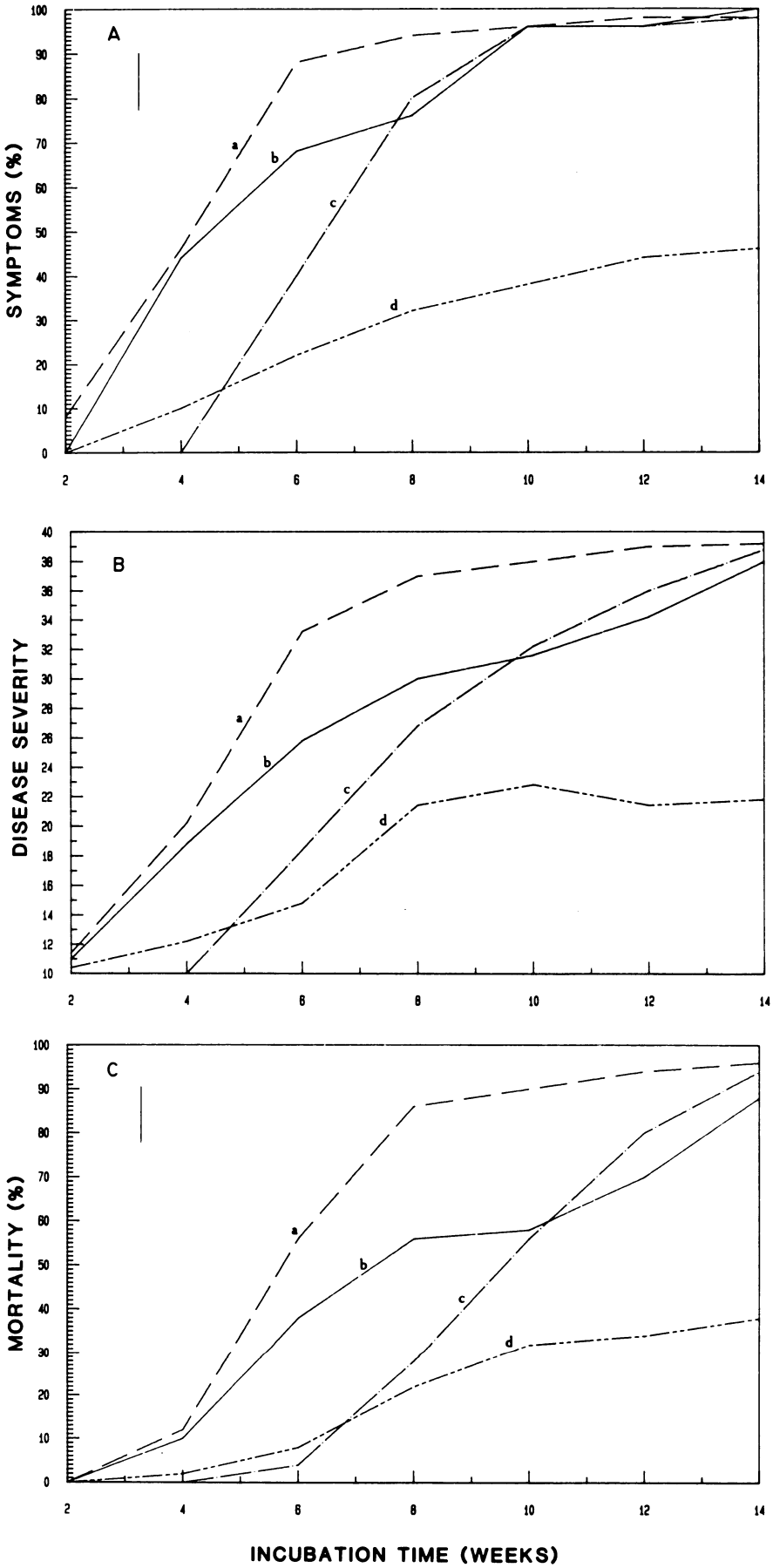

Fig. 1. Effect of inoculation method on progression of symptom expression (**A**), disease severity (**B**), and mortality (**C**) among 'Sparkle' strawberry plants inoculated with *Verticillium albo-atrum* by 4 methods; (a) macerated mycelium root dip, (b) buried colonized oat seed, (c) buried colonized vermiculite medium, and (d) conidial root dip. Each data point represents the means of 50 observations (10 plants per plot replicated five times). Vertical bars represent the LSD at $P = 0.05$.

iations of symptom expression (ranging from none to plant death) in individual tests of susceptible clones.

In this experiment, we used 4 types of inoculum: macerated mycelial mats from Czapek agar (Difco) cultures, colonized whole oat seed, colonized vermiculite substrate, and a conidial suspension. All inocula were applied to the root systems of dormant 'Sparkle' (susceptible) and 'Surecrop' (resistant) plants. Preliminary results showed that direct injection of conidia into plant crowns did not result in infection. The macerated mycelial inoculum was prepared by blending ten 5-day-old cultures of *Verticillium albo-atrum* Reinke & Berth, isolate VA-03 (a virulent strawberry isolate) from California with 1 liter of sterile distilled water. Roots of plants were dipped into the slurry, and the plants immediately were planted in the test bench. Whole oat seed was sterilized by autoclaving in 1-quart canning jars containing 650 ml oats and 100 ml of stock solution (200 ml V-8 juice and 3 g $CaCO_3$ per liter of distilled water). The oat medium was inoculated with 10 ml of isolate VA-03 conidia and incubated at 24°C for 5 days.

The vermiculite inoculum was prepared as previously described (4). Dibble holes were made in the test bench soil and 15 ml of oat or vermiculite inoculum was placed into each hole. The test plant was placed in the hole and the soil firmed around it. Conidia were washed from 5-day-old Czapek agar cultures of isolate VA-03 and diluted to 1×10^4 conidia/ml. This concentration of conidia had been adequate in preliminary tests for infection to occur. Roots of plants were dipped into the conidial suspension for 1 hr and planted immediately in the test bench. All fungal cultures were incubated under continuous fluorescent light at 22°C.

The concrete test bench, 7 m long × 1.24 m wide × 15 cm deep, was filled with a planting mix of 1 washed sand:1 Jiffy mix. The bench temperature was maintained at 20° to 25° C daily with the aid of a buried heating cable. Plants were spaced 10 cm apart in rows 10 cm apart. Inoculation treatments were replicated 5 times with 10 plants per plot arranged in a single row in a randomized complete block design. Plants were irrigated from above and fertilized and sprayed for mite control as needed. No fungicides were applied.

Plants were examined in situ and rated for disease expression at 2-week intervals until termination of the test at 14 weeks. Each plant was rated on a 1–4 scale (1 = healthy, 2 = stunted, 3 = wilted, 4 = dead) giving a possible total score of from 10 to 40 for each plot. Petioles were removed from random noninoculated control and 'Sparkle' plants exhibiting stunting or wilting and from all 'Surecrop' plants exhibiting symptoms. Petioles were surface sterilized, cut into 1–2 mm sections, placed onto potato dextrose agar and incubated for 3–4 days to permit growth of *Verticillium* from petiole sections.

Data were analyzed by analysis of variance with mean separation by Duncan's multiple range test with $P = 0.05$.

Symptoms of *Verticillium* infection were apparent 2 weeks ('Sparkle') and 4 weeks ('Surecrop') after inoculation. Among 'Sparkle' plants the most rapid symptom development (Fig. 1A) and greatest disease severity occurred with the macerated mycelium dip treatment (Fig. 1B) in which 50% mortality occurred (Fig. 1C), and an additional 32% of the living plants exhibited symptoms by 5.8 weeks. The mean disease severity index of the mycelium treatment plots was rated 32 out of a maximum of 40. Colonized oat seed induced 35% mortality and produced an additonal 31% of plants showing stunting or wilting and an overall mean plot rating of 25. Mortality among vermiculite and conidial inoculation treatments was 4% and 6%, respectively, after 5.8 weeks. The length of time for development of symptoms in 50% and 90% of plants ranged from 4.2 and 7.0 weeks, respectively (mycelium), 4.6 and 9.4 weeks, respectively (oats), and 6.6 and 9.4 weeks, respectively (vermiculite). Plants inoculated with conidia did not attain 50% symptom expression.

Plot severity ratings of 25 and 37 represented the mean severity ratings for 50% and 90% of the maximum rating attainable, respectively, in a plot. Mean plot severity ratings at the 50% and 90% levels occurred after 4.7 and 8.0 weeks, respectively, in the mycelium inoculum plots, 5.8 and 13.5 weeks, respectively, with the oat inoculum and 7.5 and 12.7 weeks, respectively, with the vermiculite inoculum. The 50% severity rating level in the conidial dip plots was not reached by 14 weeks, when the experiment was terminated.

Time to reach 50% and 90% plot mortality for plants inoculated with mycelium was 5.8 and 10.0 weeks, respectively, whereas 9.6 and 13.5 weeks were required for plants inoculated with the vermiculite inoculum to attain 50% and 90% mortality, respectively. Plants inoculated with colonized oat seed reached 50% mortality by 7.4 weeks, but 90% mortality in these plots was not reached by 14 weeks. Plants inoculated with conidia did not attain 50% plot mortality.

The percentage of plants exhibiting *Verticillium* wilt symptoms among the mycelial root dip and colonized vermiculite treatments approached 100% between 8 and 14 weeks following inoculation. Plants in the colonized oat seed inoculation treatment exhibited 100% symptom expression only at 14 weeks following inoculation. None of the treatments produced maximum plot severity ratings by 14 weeks following inoculation, as indicated in the percentage of mortality data showing 4% of the plants in the mycelial dip, 6% in the vermiculite, and 12% in the oat seed treatments remaining alive after 14 weeks.

Table 1. Recovery of *Verticillium* from 'Surecrop' plants at 2-week intervals following inoculation.

Inoculation treatment	Infection period, weeks (% recovery)						
	2	4	6	8	10	12	14
Vermiculite	0	2	2	8	14	14	14
Oat seed	0	2	2	2	6	8	10
Mycelium	0	0	0	0	8	12	14
Conidia	0	0	0	0	0	0	0
Control	0	0	0	0	0	0	0

Verticillium was recovered in all 'Sparkle' petiole isolation attempts and from several 'Surecrop' isolations. *Verticillium* was not recovered from noninoculated control plants. *Verticillium* was recovered from 'Surecrop' plants inoculated with vermiculite, oats, and mycelium but not from plants inoculated with conidia (Table 1).

Our results indicate that a rapid and reliable greenhouse bench test for *Verticillium* wilt resistance in strawberry can be feasible if certain allowances are made. For example, regardless of the inoculum type, less than 100% infection or maximum development of disease severity may occur. It is thus important to use a thoroughly randomized plot design with sufficient replications and numbers of plants per treatment to ensure the results are significant. This has been established for field test plots (1). The manner in which results are expressed, by percentage of mortality, percentage of plants with symptoms, or disease severity index is relative to the potential of the inoculum to cause disease. Infection and symptom expression in plants inoculated with *Verticillium* is a function of inoculum density and distribution in the soil (3, 5). The concentration of conidia used here was apparently too low to produce disease symptoms comparable to those produced by the other inoculum methods within the experimental time. Our preliminary work showed inoculum consisting 1×10^4 conidia/ml was adequate for infection to occur. Increasing the concentration of conidial inoculum may have produced results comparable to the oat, vermiculite, and mycelium inocula used here.

Our purpose was to determine a rapid and relatively simple technique of evaluating levels of resistance. It is apparent that the mycelium, oat or vermiculite inocula would be adequate. However, the rate at which virulence of *V. albo-atrum* is expressed, or aggressiveness, may be measured by comparing the times required to produce symptoms in plants, plant mortality, or overall disease severity expression at 50% and 90% levels. These time periods also are related directly to the inoculation method used. Here, the time differences among treatments required to achieve their 50% disease severity rating levels are relatively small; however, the 90% level was attained in the mycelium dip treatment about 4.5 weeks earlier than in the vermiculite treatment and 5.5 weeks earlier than the colonized oat seed treatment. It is evident also that disease severity ratings used in comparing levels of resistance among clones may reflect the potential of the inoculum to cause disease rather than just the genetic resistance of the host. This can be corrected partially by including a number of susceptible standard clones for comparison in evaluating test results.

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