Limited-Term Inoculation: A Method to Screen Bean Plants for Partial Resistance to White Mold

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ABSTRACT

Hunter, J. E., Dickson, M. H., and Cigna, J. A. 1981. Limited-term inoculation: A method to screen bean plants for partial resistance to white mold. Plant Disease 65:414-417.

Partial resistance to white mold (*Sclerotinia sclerotiorum*) was detected in *Phaseolus vulgaris* and *P. coccineus* with a screening technique referred to as limited-term inoculation. Pieces of autoclaved celery colonized by the fungus were attached to the stems of 2- to 5-wk-old bean plants for a limited length of time, depending on the age of the plants and the conditions under which they had been grown. When infection periods of 12, 18, and 24 hr were used, 2-wk-old plants of *P. vulgaris* 'Bush Blue Lake 47' and 'Black Valentine' were ranked as susceptible and partially resistant, respectively, and *P. coccineus* 'Tenderpod' was ranked as resistant. These rankings agree with other greenhouse and field observations. An ascosporic inoculation method gave variable results, and escapes occurred. Although high levels of resistance were not found in *P. vulgaris*, the limited-term inoculation method is sensitive and rapid enough to use to select for partial resistance. The method also has the advantage of not requiring plants to be in bloom or the production of ascospores for inoculum.

In 1973, Adams et al (4) reported that *Phaseolus coccineus* L., a species that can be crossed with *P. vulgaris* L., has some resistance to white mold, a major disease of beans caused by *Sclerotinia sclerotiorum* (Lib.) de Bary. These researchers inoculated the plants by placing an oat seed colonized by the fungus on the soil next to the stem. All *P. vulgaris* plants in their test were susceptible.

Abawi et al (3) reported resistance in *P. coccineus* and *P. coccineus* × *P. vulgaris* hybrids in a test in which blossoming plants were sprayed with a suspension of ascospores. They also found that *P. vulgaris* accessions were relatively susceptible. However, in field trials, Anderson et al (5), Coyne et al (8), and Schwartz et al (10) reported that some cultivars of *P. vulgaris* were either tolerant or resistant. In other cultivars, low incidence of disease was attributed to disease avoidance mechanisms associated with plant growth habit.

In growth chamber studies, Schwartz et al (11) found that the reactions of mature plants of susceptible and resistant cultivars could be distinguished after plants were inoculated with colonized bean blossoms. These workers concluded that a more sensitive and critically controlled environment than that used by Abawi et al (3) and Adams et al (4) was needed to detect levels of resistance of the kind observed in lines or cultivars of *P. vulgaris* in the field.

0191-2917/81/05041404/$03.00/0

Beans are difficult to screen for resistance to white mold because ascospores of *S. sclerotiorum* cannot invade green tissues unless they are provided with a nutrient source and free moisture is available on the plant surface for an extended period of time. In nature, the fungus invades green tissues mostly when these tissues come in contact with mature blossoms, which have nutrients on their surfaces (1,2,9). We report the development of a sensitive, reproducible, and rapid method of screening for resistance to white mold under greenhouse and growth chamber conditions.

MATERIALS AND METHODS

Our initial studies used the inoculation procedure of Abawi et al (3): blossoming plants were sprayed with a suspension of ascospores at a concentration of 2,000 spores per milliliter and incubated for 7 days in a greenhouse mist chamber at approximately 20–25 C. This procedure leaves contact between blossoms and green tissues to chance.

Using this method, we evaluated some of the material that Abawi and his coworkers reported to have a high level of resistance conferred by a single dominant gene. We also compared the method with a modified one in which detached, 1-day-old, fully opened blossoms were sprayed with a suspension of ascospores and placed in the axils of leaves. Altogether, 108 plants of four lines were inoculated by the leaf axil-ascospore inoculation method, and 85 plants of the same four lines were inoculated by the Abawi method. Both groups of plants were incubated in a greenhouse mist chamber at 20–25 C for 7 days.

The number of infections was not a criterion for scoring the plants; only the severity of the disease at points of infection was assessed. A subjective disease rating scale of 0 to 5 (0 = no disease; 5 = severe disease characterized by a large, water-soaked lesion covered with cottony mycelium and/or collapse of the stem) was used.

We developed a new inoculation procedure that uses small (4 × 8 mm) pieces of colonized celery petiole as inoculum. The petiole pieces were autoclaved and placed cut side down on a plate of potato-dextrose agar that had just been colonized by a rapidly growing culture of the fungus and that had not yet produced sclerotia. After incubation for 24 hr at 22 C, the celery pieces were placed on the second or third internode of 4- to 5-wk-old bean plants, and a piece of wet absorbent cotton was wrapped around the stem to hold the celery pieces in place.

Inoculated plants were incubated in a growth chamber for 48 hr with 12 hr of fluorescent light at 21 C and about 90% relative humidity. The cotton and celery were then removed. The plants were kept in a greenhouse for 3 days and then rated for disease. Plants of the same genetic background were inoculated in the leaf axils with detached blossoms sprayed with ascospores and were kept in a greenhouse mist chamber for 1 wk. Five to 40 plants in each of 10 pedigrees were inoculated by each method.

An experiment was conducted to determine if earlier removal of the celery inoculum would reduce disease severity and thereby help to differentiate levels of resistance. *P. vulgaris* cultivars Early Gallatin, Bush Blue Lake 47, and Bush Blue Lake 274 were used because of their known susceptibility; *P. vulgaris* 'Black Valentine' and *P. coccineus* 'Tenderpod' and line B-3749 were used because of observations that they possessed some resistance. Twenty-five 4- to 5-wk-old plants of each cultivar were inoculated on the second or third internode and held in a growth chamber. The cotton and celery were removed from five plants of each cultivar after 12, 24, 36, 48, and 72 hr, and the plants were immediately placed in a greenhouse. Plants were rated for disease on a 0–5 scale 6 and 10 days after inoculation.

A similar experiment was conducted with 2-wk-old plants of Bush Blue Lake 47, Black Valentine, and Tenderpod. The inoculum was removed after 12, 18, and 24 hr, but the plants were left in the growth chamber at 90% relative humidity until 48 hr after inoculation. They were then held for another 24 hr in a greenhouse before being rated as either healthy or infected.

Table 1. Incidence and severity of white mold disease in bean plants inoculated by two methods[a] with ascospores of *Sclerotinia sclerotiorum*

Bean pedigree	Number of plants in disease categories[b] 0	1	2	3	4	5
Blossoms cast naturally[c]						
7452	16	2	0	0	3	6
7453	6	0	1	2	0	0
7454	20	0	1	2	3	7
7455	9	1	0	1	1	4
Blossoms placed in leaf axils[d]						
7452	0	0	1	0	1	26
7453	7	0	1	0	3	15
7454	1	0	2	0	0	17
7455	0	0	2	0	2	30

[a] In the first method (blossoms cast naturally), blossoming plants were sprayed with an ascospore suspension; in the second method (blossoms placed in leaf axils), detached blossoms were sprayed with an ascospore suspension and placed in the axils of leaves. Plants in both groups were incubated in a greenhouse mist chamber at 20–25 C for 7 days before results were recorded.

[b] 0 = no lesions; 5 = extensive, water-soaked lesion covered with cottony mycelium and/or collapse of the stem.

[c] $n = 85$; $\bar{x} = 1.6$.

[d] $n = 108$; $\bar{x} = 4.4$.

RESULTS

Many plants of the line Abawi et al (3) reported to be resistant were susceptible in our ascosporic inoculation tests, especially when detached blossoms sprayed with ascospores were placed in the leaf axils. To determine whether the difference in results was caused by the fact that the Abawi method leaves contact between blossoms and green tissues to chance, we inoculated plants of four lines by Abawi's method and by the leaf axil-ascospore method. Mean disease ratings were 1.6 and 4.4, respectively (Table 1). These average values and a comparison of the results for each line indicate that the method of inoculation can greatly influence the frequency and severity of disease. Similar differences were found when plants derived from the cross *P. vulgaris* 'Bush Blue Lake 274' × *P. coccineus* line B-3749, previously considered resistant (3), were evaluated by these two methods.

Pieces of celery colonized by *S. sclerotiorum* and attached to the stems of 4- to 5-wk-old plants for 48 hr induced disease as effectively as blossoms sprayed with ascospores and placed in leaf axils. In eight of 10 lines, no statistically significant differences were observed between the disease incidence and

Table 2. White mold disease ratings of 4- to 5-wk-old greenhouse-grown bean plants (*Phaseolus vulgaris*) inoculated with *Sclerotinia sclerotiorum* by two methods[a]

Bean pedigree	Inoculation method: Blossom–leaf axil	Celery–internode
361520	0.7[b]	0.9
361328	1.0	1.9
361538	1.8	3.3
6985	3.2	3.2
B-3749	2.9	3.4
6986	3.6	3.3
6987	4.1	3.5
7452*[c]	4.9	3.6
BBL[d] 47*	4.9	4.4
72-112	4.7	4.8

[a] In the first method (blossom–leaf axil), a blossom sprayed with ascospores of *S. sclerotiorum* was placed in a leaf axil and plants were kept for 7 days in a greenhouse mist chamber at 20–25 C. In the second method (celery–internode), a piece of autoclaved celery colonized by the fungus was attached to the second or third internode with wet cotton. Plants were incubated in a growth chamber at 21 C and approximately 90% relative humidity for 48 hr. The cotton was then removed, and the plants were held for 3 days in a greenhouse.

[b] Average disease ratings were based on subjective disease categories from 0 (no lesions) to 5 (extensive, water-soaked lesion covered with cottony mycelium and/or collapse of the stem).

[c] * Indicates a significant difference between the inoculation methods based on a *t*-test ($P = 0.05$) analysis of each pedigree.

[d] Bush Blue Lake.

Table 3. Incidence and severity of white mold disease in 4- to 5-wk-old greenhouse-grown bean plants inoculated with autoclaved celery pieces colonized by *Sclerotinia sclerotiorum*[a]

Cultivar or line[b]	Disease rating after 6 days[c] Duration of inoculation (hr)					Disease rating after 10 days[c] Duration of inoculation (hr)				
	12	24	36	48	72	12	24	36	48	72
BBL 47 (Pv)	1.0	5.0	5.0	5.0	5.0	1.0	5.0	5.0	5.0	5.0
Early Gallatin (Pv)	3.0	4.6	5.0	5.0	5.0	3.6	5.0	5.0	5.0	5.0
BBL 274 (Pv)	3.6	5.0	5.0	5.0	5.0	4.0	5.0	5.0	5.0	5.0
Black Valentine (Pv)	1.2	2.4	4.4	5.0	5.0	2.0	3.6	5.0	5.0	5.0
B-3749 (Pc)	1.2	1.2	0.6	3.8	4.6	1.2	1.2	1.0	5.0	5.0
Tenderpod (Pc)	0.0	0.6	3.2	4.0	4.6	0.0	1.0	4.0	5.0	5.0
LSD[d] ($P = 0.05$)	2.56	1.61	1.39	0.89	0.56	2.77	2.03	1.69	0.00	0.00

[a] Celery pieces were attached to the second or third internode with wet absorbent cotton, and the plants were incubated in a growth chamber at 21 C and 90% relative humidity. Inoculum was removed after 12, 24, 36, 48, or 72 hr, and the plants were transferred to a greenhouse until disease was rated.
[b] BBL = Bush Blue Lake; Pv = *Phaseolus vulgaris*, Pc = *P. coccineus*.
[c] Average disease rating for five plants based on subjective disease categories from 0 (no lesions) to 5 (extensive, water-soaked lesion covered with cottony mycelium and/or collapse of the stem).
[d] Least significant difference.

Table 4. Incidence of disease in 2-wk-old greenhouse-grown bean plants inoculated with autoclaved celery pieces colonized by *Sclerotinia sclerotiorum*[a]

Cultivar[b]	Duration of inoculation (hr) 12	18	24
Bush Blue Lake 47 (Pv)	13/14[c]	9/11	9/9
Black Valentine (Pv)	2/13	9/13	14/14
Tenderpod (Pc)	2/12	1/11	2/12

[a] Celery pieces were attached to the stem with wet absorbent cotton, and the plants were incubated in a growth chamber at 21 C and 90% relative humidity. The celery was removed after 12, 18, or 24 hr. The plants were left in the humid growth chamber until 48 hr after inoculation, then held for 24 hr in a greenhouse before disease was recorded.
[b] Pv = *Phaseolus vulgaris*, Pc = *P. coccineus*.
[c] Number of plants infected/number of plants inoculated.

severity ratings of plants inoculated with either blossoms or celery pieces (Table 2).

Removing the celery pieces at different times from 4- to 5-wk-old plants of six cultivars indicated that disease severity could be controlled by varying the length of time the inoculum was left on the internode (Table 3). Susceptible and partially resistant cultivars could be differentiated effectively when celery inoculum was removed after 24 hr, although 12 hr was almost as good. Beyond 24 hr, separation was difficult. Black Valentine, a *P. vulgaris* cultivar, was ranked as intermediate between the three susceptible *P. vulgaris* cultivars and the two resistant *P. coccineus* lines.

Two-week-old plants could also be separated into susceptible and resistant classes by removing celery inoculum after a limited time (Table 4). In this test, nearly all the Bush Blue Lake 47 plants became infected when the inoculum was left on for only 12 hr; Black Valentine became infected within 18 hr; and Tenderpod remained healthy even when the inoculum was left on for 24 hr.

DISCUSSION

Autoclaved celery pieces were a satisfactory nutritional substitute for bean blossoms and produced more uniform disease incidence and severity than inoculation by spraying blossoming plants with ascospores. Removing the celery inoculum after a limited number of hours allowed us to detect levels of partial resistance. The ranking of Bush Blue Lake 47, Black Valentine, and Tenderpod that we obtained with this method agrees with field and greenhouse observations of the relative susceptibility of these three lines.

In studies now in progress, the concept of limited-term inoculation to detect partial resistance in beans has been substantiated with a large number of lines (*unpublished*). In this work, we are using colonized pieces of canned bean pods instead of celery and wrapping them completely around the stems of 3-wk-old plants instead of using cotton. Inoculated plants are incubated in a growth chamber at 95% or higher relative humidity or in a shaded greenhouse mist chamber.

The term partial resistance is used because plants of every line we have tested can be killed by leaving the celery or bean pod inoculum on young tissue long enough. The number of hours needed to separate susceptible from partially resistant lines depends on the conditions under which the plants have been grown and the age of the tissues inoculated. The light intensity used to grow the plants is one of the most important influences on the length of the inoculation period. For 2- to 3-wk-old plants inoculated at the first internode, 24–48 hr is usually optimal. In routine screening programs, plants of known susceptibility should be included to help determine when to terminate the test and score the plants.

Success of the test depends on high relative humidity in the growth or mist chamber. Constant 90% relative humidity is adequate when inoculum is attached to the stem with wet cotton, but 95% or higher has proved to be essential in our latest work with bean pod inoculum without the wet cotton.

Using celery or bean pod inoculum eliminates the possibility of detecting resistance associated with the blossom. However, we used this method because Schwartz et al (11) reported that senescent blossoms were colonized regardless of color or genotype. We confirmed these findings in a pilot study with blossoms from plants rated as susceptible and partially resistant.

Our method also eliminates the possibility of detecting plants with disease escape mechanisms associated with architectural characteristics. Several field trials have shown that plants with a more open canopy have less white mold because they allow better penetration of air and light into the canopy (5–7,11,12). The limited-term inoculation method can then be used to determine if lines that have less disease in field trials have true resistance or a disease escape mechanism.

Screening snap beans for resistance to white mold with ascosporic inoculum is difficult because the plants must be old enough to produce blossoms and free moisture must be present for an extended period (1,2,9). Some of the inconsistent results we obtained by spraying ascospores on blossoming plants were attributed to blossoms of different ages falling at different times onto green tissues of different maturity or not landing on green tissues at all.

Spraying detached, mature blossoms with ascospores and placing them in leaf axils allowed us to avoid escapes and standardize the time when infection was initiated. However, this method complicated screening because of variation in plant habit and time of bloom, and plants had to be grown in the greenhouse until bloom. Despite these problems, Abawi used the ascosporic inoculation method successfully to detect resistance in Black Valentine (G. S. Abawi, *personal communication*) and in *P. coccineus* line B-3749 (3).

We are now using the limited-term inoculation method to screen USDA Plant Introduction and other accessions of *P. vulgaris* and *P. coccineus* for resistance to white mold. Plants found to have intermediate levels of resistance will be crossed in an effort to accumulate favorable genes. We believe the limited-term inoculation method is sensitive enough to detect increases in resistance that may occur in the progeny as a result of this type of gene action.

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