Influence of Prebloom Disease Establishment by *Botrytis cinerea* and Environmental and Host Factors on Gray Mold Pod Rot of Snap Bean

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ABSTRACT

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Observations on the development of gray mold of snap beans in five commercial fields in the Willamette Valley of Oregon revealed that *Botrytis cinerea* colonized senescing cotyledons first. Young stem and leaf tissues also became diseased before bloom and served as within-field inoculum sources. Infected stems were the most durable prebloom inoculum source and continuously produced inoculum into the bloom period. The average number of sporulating prebloom infections varied among fields, ranging from 0.2 to 14.8 per 5-m row. Both the number of spores per plant at bloom initiation and the incidence of *B. cinerea* on blossoms at full bloom were positively correlated with the number of sporulating prebloom infections. When the number of sporulating infections before bloom was used as a predictive variable, 50% of the variation in the incidence of pod rot among fields could be explained. A multiple regression model that included the number of sporulating infections before bloom, interval between irrigations, cumulative duration of leaf wetness due to irrigation and rain, and canopy size explained 82% of the variation in percent pod rot among fields.

Additional key words: disease forecasting, epidemiology

Gray mold pod rot of snap beans (*Phaseolus vulgaris* L.), incited by *Botrytis cinerea* Pers. ex Fr., causes crop losses annually in Oregon's Willamette Valley. Infection of the pods, which reduces crop quality, occurs when colonized blossoms remain in contact

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with the pods (5,14). Consequently, disease has been controlled by foliar applications of the fungicide benomyl during bloom. In the Willamette Valley, however, the development of *B. cinerea* strains resistant to benomyl (28), coupled with lack of other efficacious compounds, has resulted in reduced fungicide use. Two unregistered compounds, iprodione and vinclozolin, show potential for gray mold control (17), but resistance to these fungicides has already been found in *B. cinerea* (8,21,23).

Disease forecasting, developed in various crops to reduce crop loss and limit fungicide use (2,22), would provide a needed management tool for control of gray mold in snap beans. To develop forecasting methods, quantitative assess-

ments of environmental and biological variables that influence the disease are made before disease develops. Forecasting models frequently include such variables as inoculum density, population of airborne spores, crop growth stage, length and frequency of moisture periods, relative humidity, and temperature (1,3,4,10,11,25).

Local inoculum sources are important in epidemics caused by Botrytis spp. (9,18,19). Further, environmental conditions favoring long periods (12-20 hr) of free moisture or high relative humidities (>94%) are required for infection of healthy plant tissue (12,15). The objective, then, is to determine the relative contributions of each component, such as environment or inoculum density, to variation in plant disease and integrate this information into a disease prediction scheme. The purpose of this study was to observe and quantify within-field inoculum sources of B. cinerea and determine the influence of these sources and environmental and host variables on gray mold pod rot.

MATERIALS AND METHODS

The development of gray mold pod rot was observed in five commercial snap bean fields. Beans were sown early (about 10 May 1981) in fields A, B, and C and late (25 June 1982) in fields D and E. Cultivar OSU 1604 or Asgrow 290 was seeded at a rate of 73–90 kg/ha in either 75- or 90-cm rows. The plantings ranged from 4 to 8 ha and were irrigated by overhead sprinklers that were handmoved across the field parallel to the rows at the grower's discretion.

Four observational plots were established in each field 4 wk after plants emerged. The plots were set out on a line across the field parallel to the rows so that all four plots would receive the same irrigation treatment. Plots were separated by 25-50 m, and each consisted of five parallel rows 5 m long and 3.8-4.5 m wide.

Inoculum sources and spore populations. The number and kinds of B. cinerea inoculum sources within the plots were recorded two or three times a week beginning 4 wk after emergence and continuing until harvest. Inoculum sources were counted only if sporulation of B. cinerea was visible on the substrate. An average plot value for the number of inoculum sources per 5-m row was obtained from the mean of the five row values for each observation date.

Conidial populations of B. cinerea on healthy bean foliage were measured on a weekly basis beginning 4 wk after emergence and continuing until harvest. In early-sown fields A, B, and C, four plants per field were sampled weekly, and in late-sown fields D and E, eight plants per field were sampled weekly. Kritzman and Netzer's (20) selective medium for B. cinerea (SBM) was used to determine spore concentration on plants. SBM was modified by deleting NaNO₃, K₂HPO₄, MgSO₄, KCl, and CuSO₄ and adding Difco potato-dextrose agar (PDA, 5 g/L), streptomycin sulfate (100 mg/L), and metalaxyl (Ridomil 2EC, $10 \mu l/L$). The roots from sampled plants were removed and the plants were weighed, then washed in 500 ml of water containing Tergitol (100 μ l/L). The wash was either plated directly or diluted from 1:2 to 1:6 before being plated on SBM. Four 0.2-ml samples per plant were plated at two wash concentrations. Plates were incubated for 72 hr at about 22 C and examined for colonies of B. cinerea. directly or diluted from 1:2 to 1:6 before being plated on SBM. Four 0.2-ml samples per plant were plated at two wash concentrations. Plates were incubated for 72 hr at about 22 C and examined for colonies of B. cinerea.

Incidence of B. cinerea on blossoms and percent pod rot. Incidence of B. cinerea on blossoms was determined at full bloom. Within each plot, 50 senescing blossoms, 10 from each row, were sampled at random from both the lower and the upper canopy. The lower canopy extended from the soil line to 20 cm above the ground, and the upper canopy was the area above 20 cm. The blossoms were individually plated onto water agar containing streptomycin sulfate (100 mg/L), incubated under a diurnal regime of 12 hr near-ultraviolet light/12 hr white fluorescent light at room temperature (about 22 C), and examined after 8-10 days for Botrytis sporulation.

The incidence of pod rot was determined by sampling plants from each

of the five rows per plot and counting all the pods over 10 cm long until 100-150 pods per row were examined. Disease incidence was measured as the percentage of pods sampled with detectable symptoms. Row values were averaged to give a plot value.

Environmental data. One electronic leaf wetness monitor (27), powered by a 12 VDC low-discharge marine battery, was placed in each field. The wetness sensors for the monitors were electrical impedance grids covered with white flat latex paint (13). The sensors were positioned in the crop canopy 20 cm above the ground and tipped at a 30° angle to ensure water runoff. The signal for the monitors was recorded on 12 VDC 0-100 microamp Rustrak Model 288 strip chart recorder (Gulton Industries, Inc., East Greenwich, RI02818). Soil moisture was measured with a Ouickdraw tensiometer (Soil Moisture Equipment Corp., Santa Barbara, CA 93105) at a soil

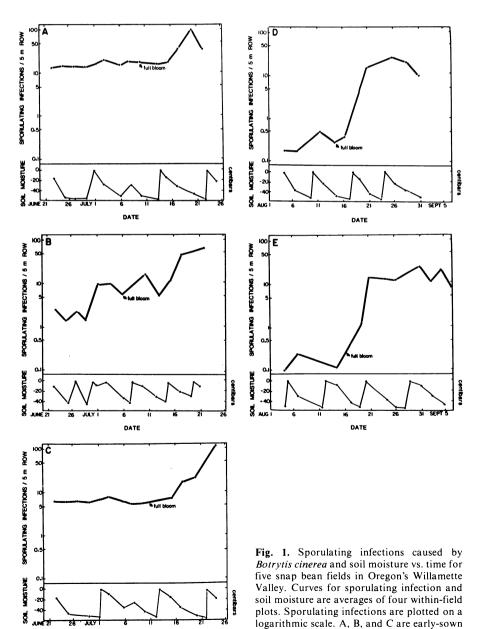
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depth of 10 cm. Two readings were made per plot per observation date. One week past full bloom, the height and width of the bean canopy were recorded. Three readings were taken on each of the five rows within each plot and averaged to give plot height and width values.

RESULTS

Within-field sources of inoculum. During the early stages of crop development, senescing cotyledons colonized by *B. cinerea* were the first inoculum source observed. In addition, stems of young bean plants and leaves near the soil surface frequently became infected. Conidia of *B. cinerea* were produced on all infected tissues. After full bloom, conidia were also produced on dehisced blossoms lying on the soil surface.

Infected stems were the most prevalent and durable inoculum source. Infections frequently girdled the stems and killed the



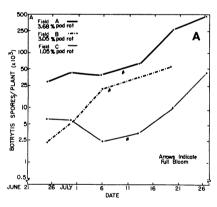
fields and D and E, late-sown fields.

plants. Sporulation on infected stems was observed 4 wk after plant emergence and continued into the bloom period on both living and dead plants.

In two of the three early-sown fields (Fig. 1A,C), 90% of the sporulating prebloom infections were on plant stems. In one field (Fig. 1A), significant stand reduction (>10%) from stem infections occurred. The durability of these stems as inoculum producers was evident from the consistent level of sporulating prebloom infections in both fields. After bloom, the increase in the number of sporulating infections to levels approaching 100 per 5-m row was primarily due to sporulation on senescent blossoms.

In the third early-sown field, leaf infections near the soil surface as well as stem infections occurred before bloom (Fig. 1B). The sporulating prebloom infection curve for this field fluctuated with the 10-cm depth soil moisture curve, which reflected time and frequency of irrigations. Fluctuations in the number of sporulating prebloom infections was due, in part, to increased sporulation on leaves during periods of high soil moisture.

The late-sown fields (Fig. 1D,E) had relatively few prebloom infections; these occurred primarily on cotyledons and stems. As with the early-sown fields, the number of sporulating infections increased after full bloom because of sporulation on senescent blossoms. The levels of



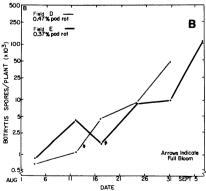


Fig. 2. Botrytis cinerea spore populations on healthy bean foliage vs. time for five snap bean fields in Oregon's Willamette Valley. Curves are mean spore populations of either four or eight plants per sampling date. Spore populations are plotted on a logarithmic scale. (A) Early-sown fields and (B) late-sown fields.

sporulating infections after bloom averaged only 30 per 5-m row in the latesown fields, whereas postbloom inoculum sources ranged from 50 to 100 per 5-m row in the early-sown fields.

Spore populations per plant. Spore populations of B. cinerea on healthy bean foliage increased in all fields from full bloom to harvest regardless of the amount of pod rot (Fig. 2A,B). Also, the number of spores per plant at harvest did not necessarily reflect the incidence of pod rot within a field. Field A, which had the highest incidence of pod rot (3.7%), also had the highest number of spores per plant at harvest $(4.5 \times 10^5 \text{ spores per})$ plant), but field E, which had the lowest incidence of pod rot (0.4%), had the second highest spore population at harvest (1×10^5) spores per plant). Nevertheless, the rankings of fields by spore populations per plant at or near full bloom for each location did correspond with the amount of pod rot at harvest (Table 1). Two early-sown fields, A and B, had significantly higher spore populations at full bloom and pod rot incidence (P = 0.05) than the other three fields.

Prebloom sporulating infections as predictive variables. Because of the relationship between spore populations

Table 1. Average number of *Botrytis cinerea* conidia per plant at full bloom and percent gray mold pod rot at harvest among five snap bean fields in the Willamette Valley of Oregon

Field ^y	Spore population per plant	Pod rot (%)
A	$6.2 \times 10^4 a^z$	3.7 a
В	$2.2 \times 10^{4} \text{ b}$	3.1 a
C	$3.8 \times 10^{3} \text{ c}$	1.1 b
D	$1.1 \times 10^{3} \text{ c}$	0.5 b
E	$1.5 \times 10^{3} \text{ c}$	0.4 b

^y A, B, C, = sown early (about 10 May 1981); D, E = sown late (25 June 1981).

² Means followed by same letter are not significantly different at P=0.05 according to the Student-Newmans-Keuls test.

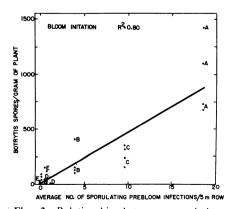


Fig. 3. Relationship between sporulating prebloom *Botrytis cinerea* infections and spore populations on healthy bean foliage at bloom initiation. Each point represents the average of four dilution plates per plant. A, B, and C are early-sown fields and D and E, late-sown fields.

on bean foliage at full bloom and incidence of pod rot at harvest, we decided that prebloom infection may be an important variable for prediction of pod rot. A predictive variable, the average number of prebloom infections per 5-m row, was computed by averaging the number of sporulating infections within each plot over the prebloom observation dates. The size of infections or amount of spore production by individual infections was not considered. The mean number of sporulating prebloom infections per field for earlysown fields A, B, and C were 14.8, 6.3, and 3.4 per 5-m row, respectively. Differences in the number of prebloom infections among the three early-sown fields were all significant (P = 0.05). The late-sown fields D and E averaged 0.4 and 0.2 sporulating prebloom infections per 5-m row, respectively. Although these differences were not significant, the number of prebloom infections in the late-sown fields was significantly lower (P = 0.05) than that in the early-sown fields.

A positive relationship was observed between number of sporulating prebloom infections and number of *B. cinerea* conidia on bean foliage at bloom initiation (Fig. 3). Number of spores per plant was transformed to spores per gram of plant to reduce variation arising from differences in size of plants sampled. The average number of sporulating infections before bloom explained 80% of the variation in spores per gram of plant at bloom initiation.

The relationship between the average number of sporulating infections before bloom and B. cinerea incidence on blossoms was positively correlated (r = 0.63) at P = 0.01 (Fig. 4). At low numbers of prebloom infections (fewer than one per 5-m row), the incidence of B. cinerea on blossoms averaged less than 10%. When the number of prebloom infections ranged from 3 to 10 per 5-m row, the average incidence of B. cinerea on blossoms was greater than 80%, and with more than 10, the incidence approached

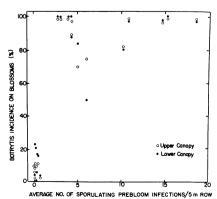


Fig. 4. Relationship between sporulating prebloom *Botrytis cinerea* infections and *B. cinerea* incidence on blossoms at full bloom for the upper and the lower canopy. Each point represents the average of 10 blossom readings.

100%. No difference in the incidence of *B. cinerea* on blossoms was noted between the upper and the lower canopy.

The average number of sporulating infections before bloom explained 50% of the variation in pod rot (Fig. 5). The relationship adequately predicted a high risk for gray mold pod rot in one of the five fields, field A. This simple regression also predicted the two low-risk fields, D and E, but did not predict pod rot for field C (6.3 sporulating prebloom infections and 1.1% pod rot) and field B (3.4 sporulating prebloom infections and 3.1% pod rot).

Contributions of environmental and host factors. A multiple regression model that included four independent variables to explain the variation in pod rot between and within fields was developed with a stepwise regression procedure. The model added the following variables in order: 1) the average number of sporulating prebloom infections per 5-m row; 2) the interval between irrigations, ie, the average number of days between irrigations; 3) cumulative leaf wetness duration (hours) during and after bloom (about 25 days) from irrigation and rain; and 4) the canopy index (canopy height × canopy width)/row width. The regression equation, which included all four variables, explained 82% of the variation in pod rot between and within fields (Table 2). The first two variables in the model, sporulating prebloom infections and interval between irrigations, explained 72% of the variation.

In tests of correlations between independent variables, the interval between irrigations was negatively correlated (r = -0.54) with leaf wetness duration (P = 0.05). In addition, both leaf wetness duration and interval between irrigations were correlated with the average number of sporulating infections before bloom (r = -0.63 and 0.69, respectively) at P = 0.05. The positive correlation between irrigation intervals and sporulating prebloom infections was a result of fields with high numbers of prebloom infections receiving fewer irrigations than fields with low numbers of prebloom infections. The canopy index, which assessed foliage density within a field, was not correlated with any of the other independent variables.

DISCUSSION

Sporulation of *B. cinerea* occurred first on senescing cotyledons and then on the stems and leaves. The stem infections fit the description of a seedling blight caused by *B. cinerea* on snap beans (29). Because *B. cinerea* generally requires an exogenous food source for infection of pod tissue (5,12,26), the colonization of senescing cotyledons may be involved in the initiation of the stem infections. Conversely, the leaf infections near the soil surface observed in field C did not appear to result from contact with an

exogenous food base. The sand content of this field was very high (78% sand, 19% silt, 3% clay), and the fungus may have entered the tissue via wounds created by leaves in contact with sand particles (14).

The source of primary inoculum of B. cinerea that initiates prebloom disease development in the Willamette Valley is still unresolved. Other researchers have found that B. cinerea can survive for extended periods in the field as sclerotia, mycelia, and possibly conidia (6). In the Willamette Valley, sclerotia of B. cinerea frequently form on crop debris and can overwinter and produce conidia (16). Although Polach and Abawi (24) have observed Botryotinia fuckeliana, the perfect stage of B. cinerea, in bean fields in New York, apothecia have not been reported in Oregon despite routine surveys made by pest management specialists.

Numbers of prebloom infections of B. cinerea were significantly greater in the early-sown fields than in the late-sown fields. Postemergence environmental conditions were cool (daily mean temperature = 13.8 C) and rainy (daily mean precipitation = 4.5 mm) as earlysown plantings emerged. In contrast, plants in the late-sown fields, where incidence of prebloom infections was low, emerged under warm (daily mean temperature = 18.4 C) and dry (daily mean precipitation = 0.08 mm) conditions. The cool, wet conditions may have favored prebloom disease development in the early-sown fields (5,14).

In terms of practical disease forecasting, the quantification of sporulating prebloom infections provided an estimate of the amount of inoculum on the bean foliage at floral initiation. The regression line of plant spore populations on number of sporulating prebloom infections (Fig. 3) nearly passed through the origin. We interpreted this to mean that prebloom infections were responsible for most of the inoculum on bean foliage. A similar relationship was observed when incidence of B. cinerea on blossoms was plotted against sporulating prebloom infections (Fig. 4); as few as 2.8 prebloom infections resulted in 100% incidence, however.

Because of the positive relationship between plant spore populations at full bloom and amount of pod rot at harvest (Table 1), we concluded that the arrival of inoculum at the infection court, ie, the senescent blossoms, during the bloom period is important in disease development. After bloom, inoculum was produced by colonized senescent blossoms lying on the soil surface; however, this postbloom inoculum production may not be important in pod rot development. We presented evidence for this in studies of spore dispersal gradients of B. cinerea and pod rot disease gradients from point inoculum sources in snap beans (18). Because of production of secondary inoculum, spore dispersal gradients were significantly flatter at harvest than at full bloom, whereas gradients for pod rot incidence at harvest did not flatten when compared to incidence of B. cinerea on blossoms at full bloom. From the standpoint of disease forecasting, this finding supports the concept that assessment of sporulating prebloom infections is critical in predicting gray mold pod rot potential.

Principles of plant disease epidemiology dictate that factors other than inoculum alone will influence an epidemic. Though our study was concerned primarily with the role of prebloom infections in predicting gray mold pod rot, additional variables are important in disease development. For example, the interval between irrigations explained a substantial proportion of the variation in amount of

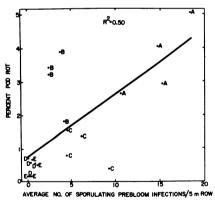


Fig. 5. Relationship between sporulating prebloom *Botrytis cinerea* infections and percent pod rot at harvest. Each point represents the average of 500-700 pod disease readings. A, B, and C are early-sown fields and D and E, late-sown fields.

Table 2. Stepwise multiple regression analysis of biological and environmental variables to explain variation in percent gray mold pod rot

Regression model ^a	Cumulative coefficient of determination	$P^{ m b}$
%PR = 0.77 + 0.19S	0.50	0.001
%PR = 4.60 + 0.30S - 0.60I	0.72	0.001
%PR = 0.02 + 0.32S - 0.46I + 0.06W	0.79	0.025
% PR = -7.12 + 0.40S - 0.41I + 0.14W + 0.07C	0.82	0.075

^a%PR = percent gray mold pod rot; S = average number of sporulating prebloom infections per 5-m row; I = interval between irrigations; W = cumulative leaf wetness during and after bloom due to irrigation and rain; C = canopy index.

^bProbability that regression coefficient of variable entering model is equal to zero with previous variables in model.

pod rot. The combined R^2 value of 0.72 reflects the importance of irrigation and number of prebloom infections in disease development. Furthermore, the cumulative leaf wetness duration during and after bloom due to irrigation and rainfall and the postbloom canopy index also contributed significantly to disease development. These latter two variables, coupled with irrigation, influence the microclimate under which germination, sporulation, and infection occur (7.15).

Validation and refinement of the variables in the multiple regression model are still required. The limited amount of data used in the model, ie, five fields in 1 yr, and the intercorrelations among independent variables reduce the amount of confidence that can be placed on the regression coefficients obtained. Also, for prediction of disease development, additional research is required to assess or estimate contributing variables before or during the initial stages of bloom when decisions to apply fungicides are made.

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