The Potential of Four Dry Bean Cultivars to Serve as Sources of *Pseudomonas phaseolicola* Inoculum

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ABSTRACT

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Seedlings of the cultivars California Light Red Kidney, Redkloud, Redkote, and HLR (a breeding line with a high degree of halo blight resistance) were inoculated with *Pseudomonas phaseolicola* and transplanted into small plots containing healthy snap bean plants to assess the ability of inoculated plants to serve as sources of inoculum in the field. The percentage of transplants around which snap bean plants showed symptoms of halo blight was 80% for the cultivar California Light Red Kidney and 40% for the cultivar Redkloud. No significant difference was found among the number of secondary infection centers that developed in plots with Redkote and HLR transplants and in control plots without transplants. There was a direct relationship between a cultivar's susceptibility to halo blight and its potential to serve as an inoculum source after artificial inoculation.

Halo blight of beans (*Phaseolus vulgaris*) caused by *Pseudomonas phaseolicola* (Burk.) Dows. is an important disease where growing conditions are cool and rain is frequent. Traditionally, the chief means of control for this disease in northeastern United States has been the use of pathogen-free seed produced in the semiarid West (6,8). Growers in New York recently have been growing cultivars with moderate resistance to halo blight from seed produced in New York. Growers of susceptible bean cultivars are concerned that plants grown from such seed might serve as sources of inoculum for susceptible cultivars growing nearby.

Our study compares the potential of four dry bean cultivars with differing degrees of susceptibility to halo blight to serve as sources of inoculum for susceptible snap bean plants.

MATERIALS AND METHODS

The spread of *P. phaseolicola* from four dry bean cultivars was investigated by transplanting inoculated plants into small plots of susceptible snap bean plants. The snap bean plants were then monitored for the appearance of halo blight symptoms throughout the growing season.

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Each plot consisted of four rows 3.5 m long and 0.5 m apart seeded at a rate of 25 seeds/m with the snap bean cultivar Provider (Harris Seed Co., Rochester, NY 14624), which is highly susceptible to halo blight. Plots were separated by a 2.5-m space, in which one double row of vining peas (*Pisum sativum*) was planted to reduce interplot contamination. No other plantings of beans were in the immediate vicinity of the test plots.

Four dry bean plants inoculated with *P. phaseolicola* were transplanted into each plot 10–14 days after they had been inoculated. At this time, the snap beans had fully expanded unifoliolate leaves but had not yet produced fully developed trifoliolate leaves. The following cultivars, chosen for their different degrees of susceptibility to halo blight and listed in decreasing order of susceptibility, were used: California Light Red Kidney (CLR Kidney), California certified seed; Redkloud, New York foundation seed grown in Idaho; Redkote, New York certified seed; and HLR, a highly resistant advanced generation breeding line (7). (In this paper, the breeding line HLR is included under the term "cultivar.") Twenty-five small plots of snap bean plants were planted; for each cultivar there were five replicate plots, each to receive four transplants of the given cultivar, and five control plots without transplants. These plots were arranged in a completely randomized design, except that plots with CLR Kidney transplants, the most susceptible cultivar (4), were located together at the downwind edge of the blocks to minimize the possibility of bacteria spreading from these plots to other plots.

Plants to be transplanted were inoculated by spray-infiltrating unifoliolate leaves 24–48 hr after they unfolded; a suspension containing 10^3 *P. phaseolicola* cells/ml was used, following the procedure of Schuster (5). Inoculum was prepared by diluting 18-hr nutrient broth cultures of *P. phaseolicola* (MF strain, 4) in sterile distilled water. The inoculated plants were incubated 10–14 days in growth chambers at 18 C night temperature and 22 C day temperature; day length was 16 hr with an intensity of 1,860 lux. In addition, after 10 days the first trifoliolate leaf of half the plants of each cultivar was inoculated with a suspension of 10^2 *P. phaseolicola* cells/ml. Inoculation produced about 120 lesions on CLR Kidney, 80 on Redkloud, 20 on Redkote, and none on HLR at the time of transplanting. Two plants inoculated on their unifoliolate leaves only and two plants with the double inoculation were transplanted into each test plot.

The experiment was done in two fields near Ithaca, NY, during the 1978 growing season. One field received 1.2–2.5 cm water per day from overhead sprinklers operated for 0.5 hr twice daily at 0730 and 2000. Inoculated dry bean plants were transplanted into this field from 15–20 July. The other field, which was not irrigated, received transplants from 28 June to 1 July. The inoculated plants were transplanted 2 m apart, two plants per row in the middle two rows of snap bean plots. They were staggered by planting one 0.5 m from the end of row 2 and another 1 m from the end of row 3.

Plots were examined every 3 days for the appearence of halo blight symptoms. Isolations from symptomatic tissue were made on medium B of King et al (3). The

number of inoculated transplants around which symptoms of halo blight were seen on the snap bean plants was totaled for each cultivar and subjected to a one-way analysis of variance at the 1% significance level.

RESULTS

The first symptoms of halo blight were observed at the irrigated location 15 days after transplanting. These symptomatic snap bean plants were in the plots containing CLR Kidney transplants (Fig. 1). New locations of secondary spread of *P. phaseolicola* were observed for the next 30 days, with 16 of the 20 CLR Kidney transplants eventually having symptomatic snap bean plants around them.

In the plots containing inoculated Redkloud, halo blight symptoms were first observed on Provider 33 days after transplanting. Infection around individual Redkloud plants continued to appear for the next 20 days, with eight of the 20 transplants serving as inoculum sources.

Symptoms developed on Provider near only one Redkote transplant 40 days after transplanting. A second area of secondary spread was observed 46 days after transplanting; this was not centered around a transplant. Although this infection probably did not originate directly from inoculum provided by a transplant, it was used in the statistical treatment of the data. No symptoms were observed in the plots containing HLR transplants. Plants in one of the control plots without transplants showed symptoms 46 days after transplanting was done in other plots.

Due to senescence of the snap beans, observations ceased on 11 September, 54 days after the inoculated plants were transplanted. There was no significant difference in the total number of secondary infections originating from transplants inoculated on unifoliolate leaves only and those originating from twice-inoculated plants, although in all cases the first transplant to have symptomatic snap beans around it had been inoculated on its unifoliolate leaves only. These plants were put into the field 4 days before the twice-inoculated ones. In all cases, isolation from symptomatic tissue onto medium B of King et al yielded a fluorescent bacterium comparable in colony appearance to *P. phaseolicola*.

No halo blight symptoms were observed at the nonirrigated location. There was little if any further disease development on the inoculated plants. Even on the CLR Kidney, the inoculated leaves dried up and no new symptoms were observed.

DISCUSSION

The appearance of plants showing halo blight symptoms surrounding 80% of the transplanted CLR Kidney plants demonstrates the ability of infected plants of this cultivar to serve efficiently as inoculum sources. These results are in agreement with previous studies (4) that indicate the high degree of susceptibility of CLR Kidney to halo blight as shown by symptom expression and bacterial growth in vivo. The higher rate of bacterial multiplication and the larger final bacterial population in this cultivar relative to the other cultivars (M.J. Katherman, R.E. Wilkinson, and S.V. Beer, *unpublished*) probably account for the earlier appearance of symptomatic snap bean plants in the plots containing CLR Kidney. Snap bean infections in these plots occurred 22 days earlier than those seen around Redkloud transplants and 29 days before spread was observed from the Redkote transplants. Preliminary studies indicate, however, that viable bacteria are present even in HLR, the most resistant cultivar, at least 20 days after inoculation, although in lower numbers than in CLR Kidney.

Redkloud, representing an intermediate degree of susceptibility, also has the potential to serve as a source of inoculum of *P. phaseolicola* under conditions favorable to the disease. The somewhat later appearance of halo blight around the transplants of this cultivar reflects its lesser susceptibility to the disease.

The total number of inoculum centers around which symptomatic snap beans were seen in the plots containing Redkote transplants and those containing HLR transplants did not differ significantly from the number for the control plots without transplants. Although Redkote is more susceptible to halo blight than HLR, based on symptom severity and bacterial growth in inoculated leaves (4; M.J. Katherman, R.E. Wilkinson, and S.V. Beer, *unpublished*), the difference was not reflected in this field experiment. The degree of resistance in Redkote apparently reduces its ability to serve as an inoculum source. The possibility remains, however, that infected plants of this cultivar can lead to secondary spread of *P. phaseolicola*, as evidenced by the symptomatic snap bean plants seen around one of the Redkote transplants. Little primary inoculum is necessary to initiate an epidemic of halo blight in a highly susceptible cultivar. Walker and Patel (6) observed that 30 diseased plants per hectare (which represents little more than 0.01% of the total seed normally planted) were sufficient to initiate infection that led to the decimation of a snap bean planting. Therefore, even a very small number of effective sources of inoculum, such as seen for Redkote in our experiment, might have great significance in commercial production.

On the basis of observations of plots without inoculated transplants, plot-to-

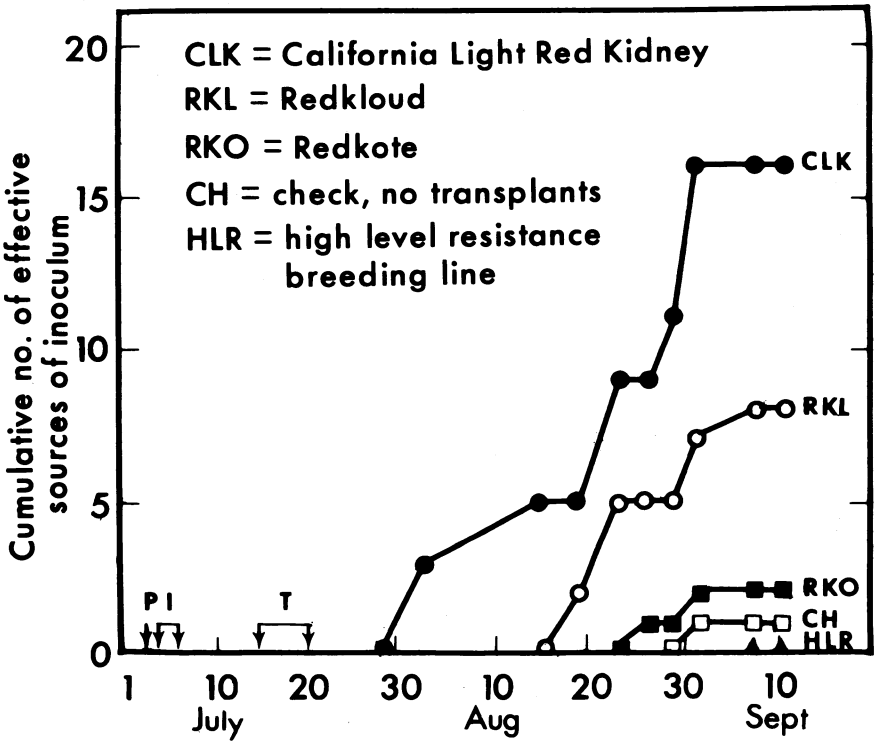

Fig. 1. Spread of *Pseudomonas phaseolicola* from inoculated, transplanted dry beans of four cultivars to surrounding snap bean plants in the field under sprinkler irrigation. Dates of inoculation (I) and transplanting (T) of the dry bean plants and planting date of the snap bean cultivar Provider (P) are indicated. The cumulative number of transplants around which symptomatic snap beans were observed (effective source of inoculum) is significantly greater for California Light Red Kidney (16) than for Redkloud (8), which is significantly more than the remaining three plot types ($P = 0.01$). The final number of infection centers in check (control) plots and in plots with Redkote and HLR do not differ significantly.

plot spread of *P. phaseolicola* or infections from inoculum from other sources did not materially interfere with observations of spread from inoculated transplants.

The inoculated transplants in this study were intended to mimic plants grown from seed carrying the pathogen. The inoculated plants probably had a greater potential as sources of inoculum than would naturally infected plants. These results do not measure the extent to which seed transmission can occur in cultivars with some resistance, and this bears directly on their potential to serve as sources of inoculum. In another study, however, we demonstrated transmission of *P. phaseolicola* from seed produced on Redkloud plants that were inoculated several times during the growing season (R.E. Wilkinson and M.J. Katherman, *unpublished*). Similar studies have not been done with Redkote or HLR.

The experimental conditions used were especially favorable for the dissemination of *P. phaseolicola* and development of halo blight (2). These included twice-daily sprinkler irrigation to aid in inoculum dispersal and give favorable conditions for bacterial multiplication. The lack of development of halo blight in the nonirrigated field may be attributed to dry weather when secondary spread of inoculum was expected. In the Ithaca area during that period, only 5.92 cm of rain fell (3.35 cm less than the 24-yr average), 2.4 cm of that falling during one 24-hr period (1). Walker and Patel (6) emphasized the importance of rain for the dissemination of this pathogen.

It should be emphasized that this study only suggests that a particular cultivar has the potential to serve as a halo blight inoculum source; whether under field conditions a particular cultivar actually does so is determined by weather and cultural conditions. We conclude that infected plants of both CLR Kidney and Redkloud have the potential to serve as sources of inoculum for healthy plants when environmental conditions favor dissemination and disease development, whereas Redkote and HLR show little potential. The ability of dry bean cultivars to serve as inoculum sources and their susceptibility to halo blight appear to correspond closely.

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