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Comparing 2 Inoculation Techniques for Evaluating Resistance in Beans to *Xanthomonas campestris* pv. *phaseoli*

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Abstract

Two inoculation techniques were compared for efficacy in screening resistance to common bacterial blight (CBB) caused by *Xanthomonas campestris* pv. *phaseoli* (Xp). Bean cultivars Jules, BAT 93, ICA L24, BAT 47, BAT 76, and Porrillo Sintetico, with known reactions to the blight in Colombia were inoculated in the field and in the greenhouse with Colombian isolate Xp-123. A razor blade procedure and a modification of it using surgical blades were compared. In all cases plants were inoculated in the first trifoliate 31 days after planting and evaluated 8 and 12 days after inoculation. In the field and greenhouse, the ranking of the cultivars based on their CBB reactions was the same with both inoculation techniques. However, the surgical blade was less effective in eliciting the expected CBB reaction from cultivars ranked as intermediate. The uniformity of CBB reaction was greater with the surgical blade procedure. It was also easier to use and faster. Both procedures are excellent methods for the evaluation of CBB resistance in beans in the field and both allow the simultaneous evaluations of other characters such as adaptation, architecture, and resistance to other diseases.

Introduction

Common bacterial blight (CBB) caused by *Xanthomonas campestris* pv. *phaseoli* (Smith 1897) Dye and Wilkie 1978b (ISPP List 1980) [*Xanthomonas phaseoli* (Smith) Dowson] is a major disease of dry beans.

The disease is widely distributed in both temperate and tropical bean growing regions where it can cause considerable reductions in yield and in seed quality (13). The pathogen is seed transmitted and can attack leaves, stems, pods, and seeds. In the U.S.A., the use of clean seed produced in the semiarid regions of the west and other cultural practices and chemical controls are important components of the disease management strategy (13, 14). In the tropics, particularly where subsistence agriculture may prevail, such practices are difficult to utilize, thus the use of resistant cultivars provides the most adequate and practical method of CBB control (10).

An important initial step in the generation of disease resistant cultivars is the development of a reliable procedure for inoculating plants with *pathogens* artificially. Some of the criteria for choosing the inoculation procedure should be effectiveness in inducing distinct responses in resistant and susceptible cultivars, repeatability, and ease of using in the field where large numbers of plants may be evaluated. In addition, the procedure should be rapid, uniform, and easy to learn and implement.

In the past, several procedures have been utilized, including: A, the pricking of the stem with a needle (4) or a scapel (2) previously immersed in a bacterial suspension; (B) rubbing leaves with a bacterial suspension mixed with carborundum (3); C, spraying entire leaves with a bacterium suspension at high pressure (2, 8); D, vacuum leaf infiltration (9); E, perforation of leaves with a multiple needle inoculator (1, 7); and F, clipping of leaves with contaminated scissors (6, 10).

At CIAT, a modification of the scissors leaf-clipping procedure as reported by Webster (10) was used initially. Webster inoculated half primary or trifoliate leaves by cutting about 1.5 cm into the leaf with scissors previously dipped in inoculate. A second cut was also made about 2 cm toward the tip of the leaf (10).

In a modified procedure, two razor blades were mounted 2 cm apart and parallel to each other on a wooden handle. Trifoliate leaves were then placed on a sponge soaked in a bacterial cell suspension and cut in two areas as shown in Fig. 1. A modification of this "razor blade method" called "the surgical blade" procedure, was developed later at CIAT.

The objectives of the present study were: To report the surgical blade method as a new inoculation procedure; and to compare the efficacy of the razor blade and surgical blade procedures, the two inoculation techniques utilized at CIAT for the evaluation of common bacterial blight resistance in beans.

Materials and Methods

A randomized block design with five replications was employed. The field experiment was conducted at the CIAT experimental fields near Palmira in 1981. Six bean cultivars, representing a cross section of the different host reactions to the CBB pathogen were used (Table 1). The six included 2 resistant materials, Jules (P698) and BAT 93; 2 intermediates, ICA Line 24 and BAT 47; and 2 susceptibles, Porrillo Sintético (P566) and BAT 76. Twenty seeds were planted per 2 m row plots with rows spaced 60 cm apart. The field was isolated from other bean plots.

The experiment was also conducted in the greenhouse and in a growth room. In the last two locations 2 plants per pot constituted a plot. Field plots were planted January 13, 1981 and greenhouse and growth room planting was conducted the next day. Inoculation was done on February 6.

Inoculation with the razor blade has been described previously (5). In the surgical blade procedure, a bacterial cell suspension was placed in a plastic bottle equipped with a rubber stopper with two small round perforations in which pieces of sponge were placed to allow the inoculum to pass slowly.

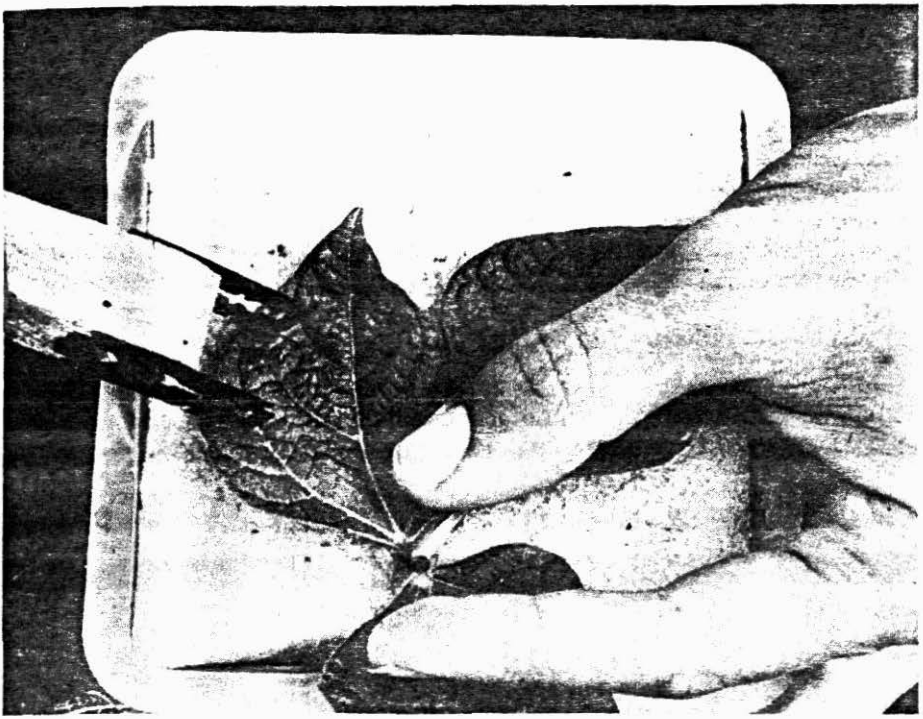

Fig. 1. Razor blade equipment used to inoculate bean plants with the common bacterial blight pathogen.

Two steel surgical blades were placed in the rubber stopper next to the perforations. Both the rubber stopper and blades were enveloped in a piece of cheese cloth to holdthem in place. Leaves were placed on a sponge in a tray and stabbed with the blades; inoculum flowed through the holes in the

Table 1. Known reaction of selected bean cultivars to *Xanthomonas campestris* pv. *phaseoli.*

Cultivar	Origin	Reaction[a]
Jules (P 698)	U.S.A.	R
BAT 93	CIAT	R
ICA Linea 24	ICA-Colombia	R - I
BAT 47	CIAT	I
Porrillo Sintetico	El Salvador	S
BAT 76	CIAT	S

[a] Disease (Common bacterial blight) reaction; R = resistant; I = intermediate; S = susceptible; based on 1-5 scale, 1-2 = resistant; 3 = intermediate; 4 and 5 = susceptible.

stopper, saturating the cheesecloth and blades (Fig. 2). In all cases, the inoculum potential consisted of 5 x 10^7 bacterial cells/ml of water. One trifoliate leaf was inoculated per plant. All plants in the row were inoculated. Rows of control plants were inoculated separately with both procedures using sterile distilled water. Isolate XP 123 collected in Palmira was used.

The maximum and minimum temperatures were: field, 30° and 19°; greenhouse, 30.8 and 19.9°; growth room, 22.3 and 18.2°. The maximum and minimum relative humidities were: field, 91.5 and 46°; greenhouse, 98 and 46°; growth room, 93 and 44.2°. In another treatment, plants were placed in a greenhouse bench until inoculation time; subsequently, they were placed in a humidity chamber with 100% RH for two days. Plants were evaluated 8 and 12 days later in the field and growth room. Only one evaluation, 10 days after the inoculation, was possible in the greenhouse, due to early senescence of some of the trifoliates. Bacterial blight severity was rated using a 1:5 scale (Fig. 2 and Fig. 3).

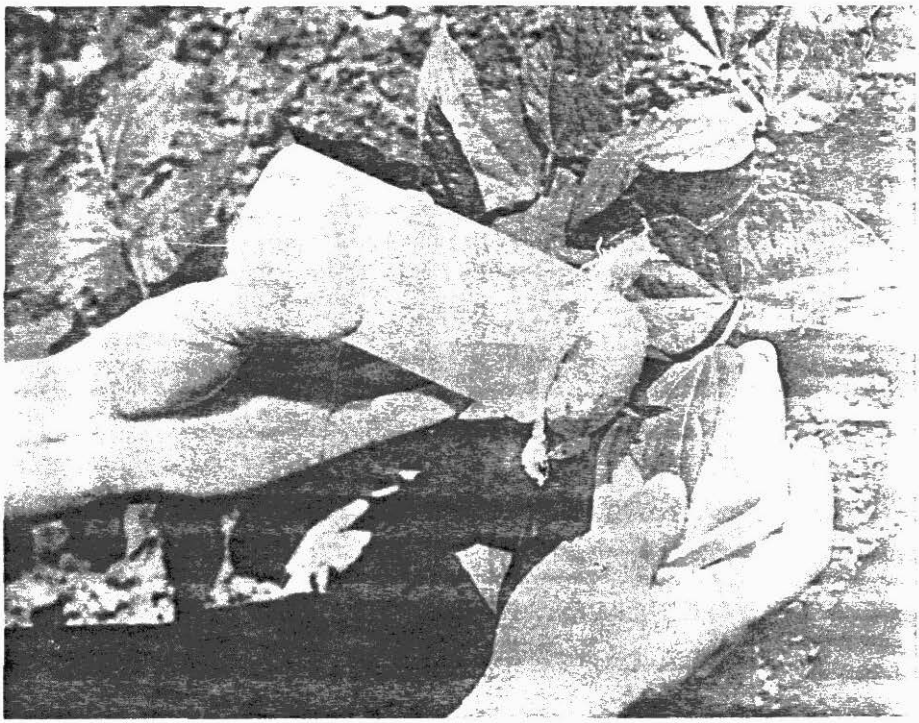

Fig. 2. Surgical blade inoculation equipment. Leaves are placed on a sponge saturated with inoculum. The plastic bottle also contains inoculum. The leaf is punctured with two blades. Inoculum flows through two holes in the stopper next to the blades. The holes are fitted with sponges. Flow from the bottle keeps the blades and cheese cloth holding sponges in place saturated with inoculum.

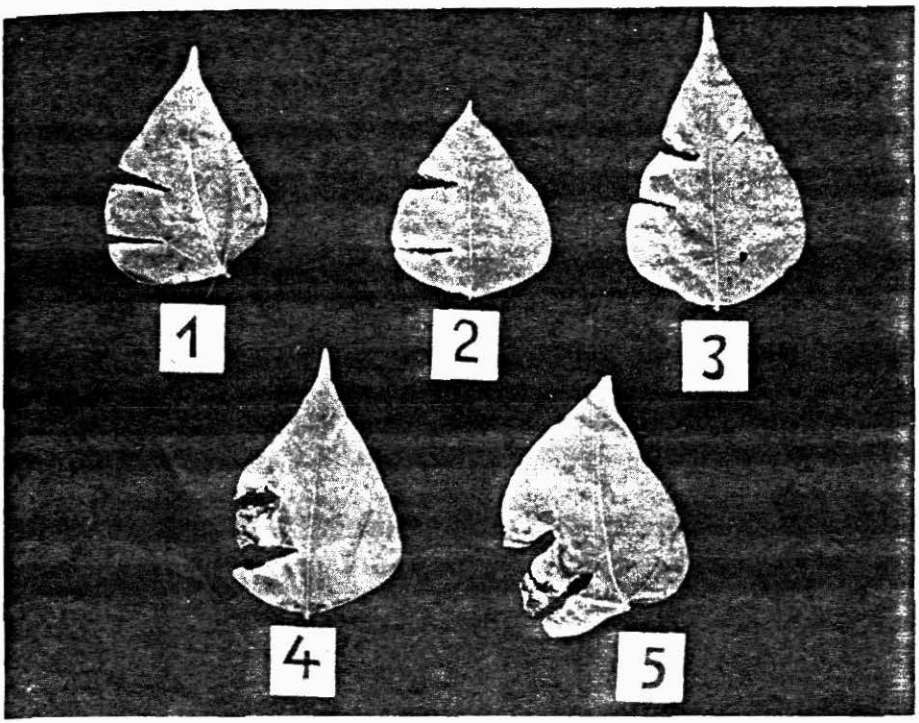

Fig. 3. Common bacterial blight severity scale used for razor blade inoculation procedure.

Results and Discussion

The objective was to compare two inoculation procedures for the evaluation of CBB resistance in beans. One criteria was that the procedure should eliminate the possibility of selecting scapes as resistant. In addition, the procedure should be rapid, to allow the inoculation and evaluation of large populations in the field. It should be easy to use, uniform, and should allow simultaneous evaluation in the field for CBB resistance and other characters such as pod load, architecture, and reaction to other diseases such as rust and bean common mosaic virus.

During the first field evaluation, ranking of the cultivars based on their CBB reaction was the same with both inoculation methods (Fig. 4); however, the CBB reaction was lower than the expected disease reaction from these cultivars under local conditions for both inoculation methods. Differences in CBB scores were not significant during the first evaluation between the two procedures. During the second field evaluation, however, there were significant differences in CBB scores between the razor blade and surgical blade procedures. The razor blade plus bacteria treatment induced a wider range of reactions and distinguished better between intermediate BAT 47 and resistant cultivars (Fig. 5). The surgical blade procedure was less effective in eliciting the expected reaction from cultivars ranked as intermediate.

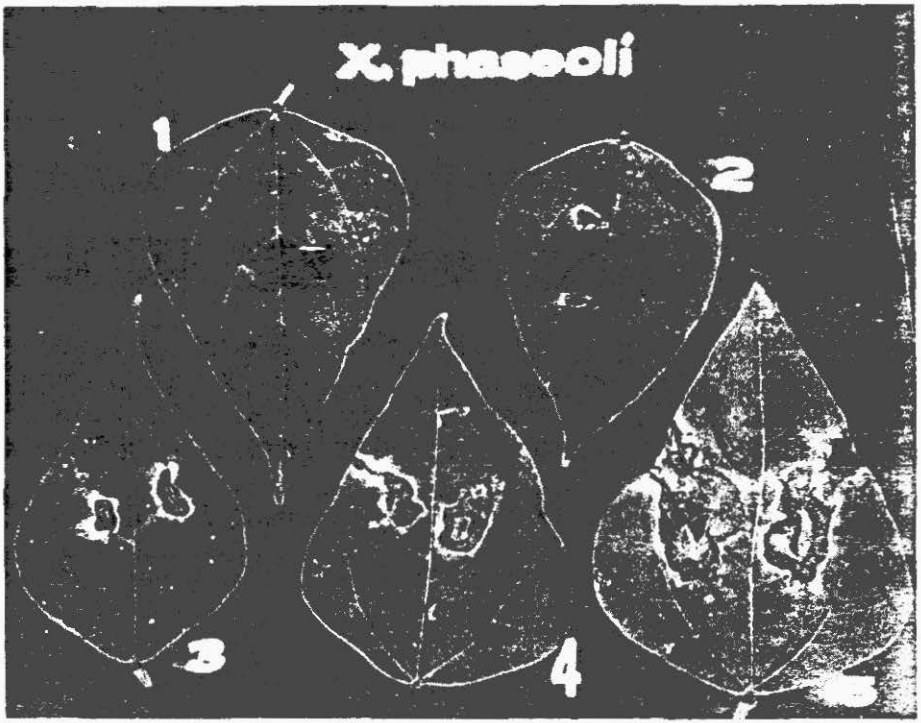

Fig. 4. Common bacterial blight severity scale used for the surgical blade inoculation procedure.

The separation of the cultivars into resistant, intermediate, and susceptible categories was more definite and clear with the razor blade procedure. A second evaluation 13 days after inoculation, was necessary before the known reaction to the bacteria by the cultivars used could be observed. The controls, where water rather than a bacterial cell suspension was used as the inoculum, did not cause any reaction.

The evaluation in the greenhouse yielded results similar to those observed during the second evaluation in the field. There were significant differences in CBB scores between the two procedures (Fig. 6). Even though the ranking of the cultivars based on their CBB reaction was similar with both procedures, the segregation of the cultivars into their known resistant, intermediate, and susceptible field reaction groups was much wider with the razor blade procedure than with the surgical blade. ICA Line 24 generally has an intermediate to resistant CBB reaction in the field; however, under greenhouse conditions, when inoculated with either procedure, its reaction is relatively more susceptible (Fig. 5 and 6). We have observed that many determinate cultivars (CIAT Type I) have similar CBB field reaction to that of ICA L 24, possibly due to the foliage type. The foliage, characterized by larger and thicker leaves, may be altered under greenhouse conditions, causing the difference in CBB reaction between the field and greenhouse.

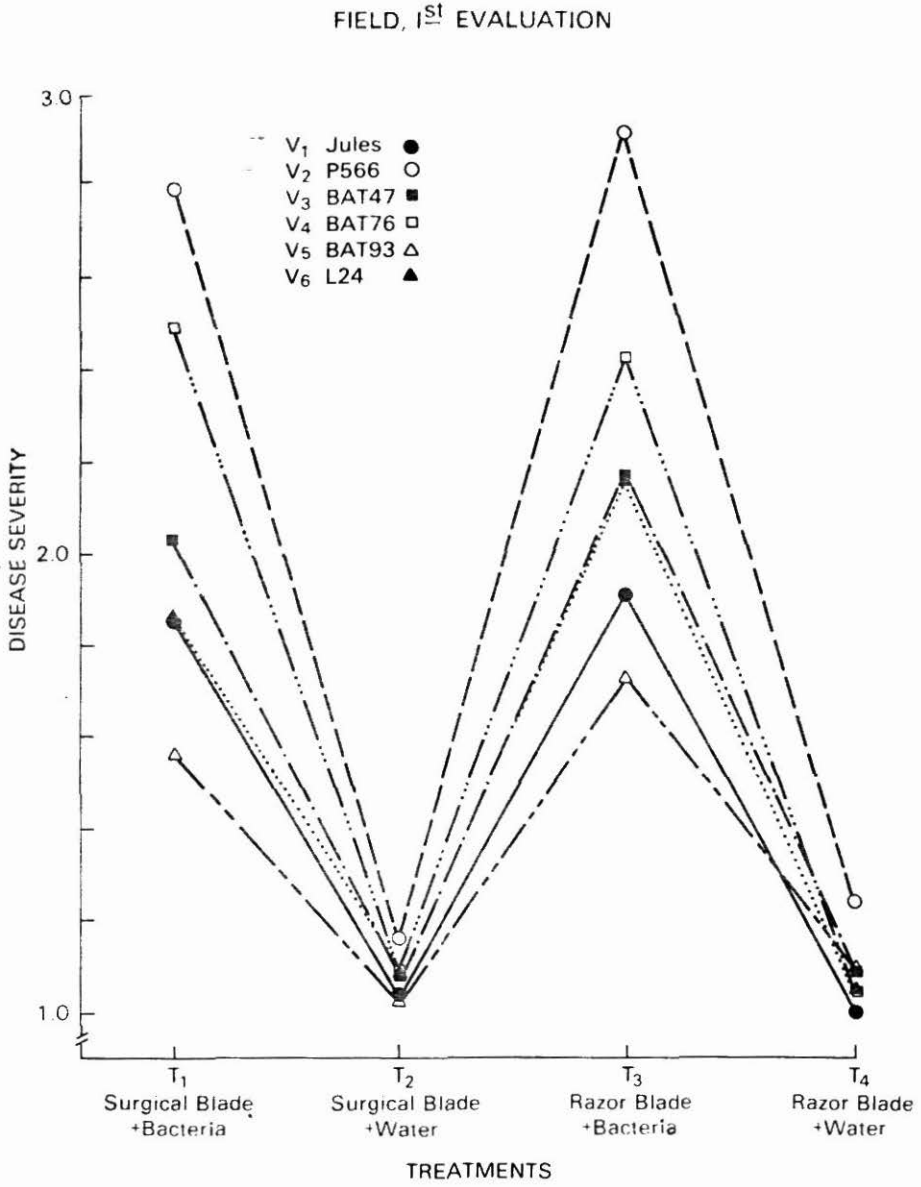

Fig. 5. Common bacterial blight reaction of six bean cultivars inoculated in the field with the razor blade and surgical blade procedures and evaluated eight days after inoculation.

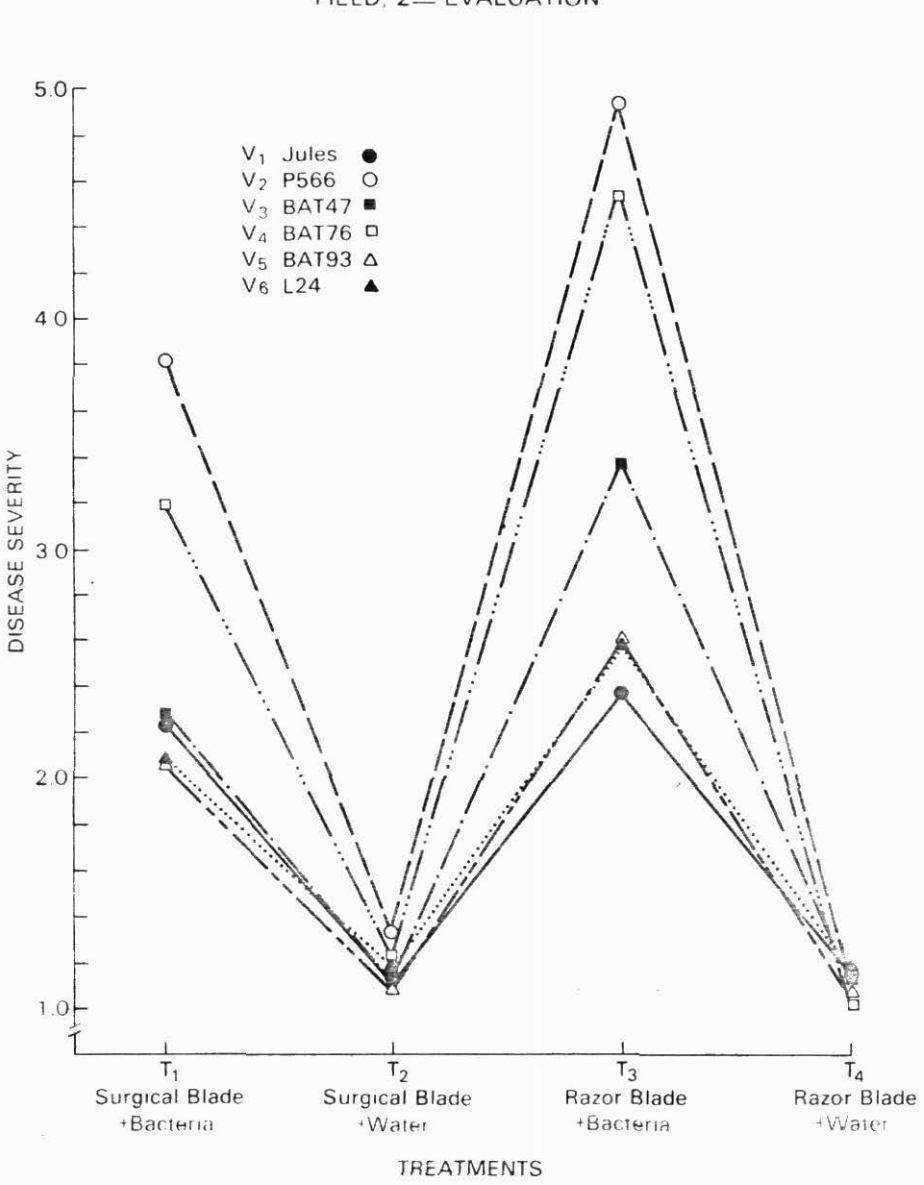

Fig. 6. Common bacterial blight reaction of six bean cultivars inoculated in the field with the razor blade and surgical blade procedures and evaluated eight days after inoculation.

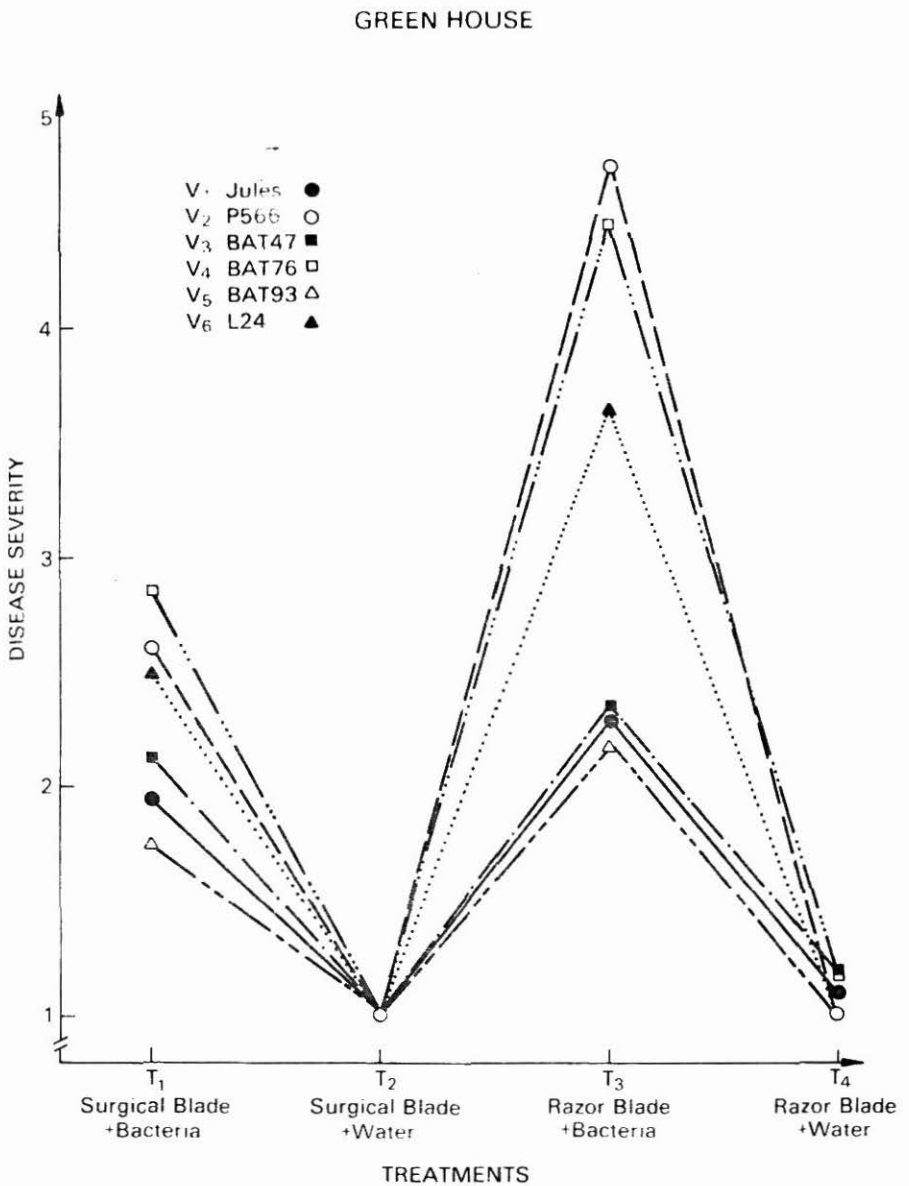

Fig. 7. Common bacterial blight reaction of six bean cultivars inoculated in the greenhouse with the razor blade and surgical blade procedures and evaluated ten days after inoculation.

The treatment in which cultivars maintained in a greenhouse were submitted to a 48 h period of high humidity in a humidity chamber after inoculation yielded similar results to those in which the cultivars were maintained continually in the greenhouse. In the humidity chamber treatment ICA Line 24 had a susceptible reaction, contrary to results found in the field. In general, the disease severity in this treatment was lower for all cultivars due, perhaps, to the lower temperature of the humidity chamber. For methods of inoculation which introduce the pathogen into a wound, the pathogen need not survive externally on the host, hence a humidity treatment is probably not necessary.

In the growth room disease symptoms were very slow to develop. Here the temperature during the entire period was lower than in the greenhouse and field. The ranking of the cultivars based on their CBB reaction was similar to that of the field and greenhouse. The growth room and humidity chamber treatments offered conditions less adequate than the field and greenhouse for CBB screenings.

In general, the razor blade method of CBB inoculation elicited higher disease severity in the susceptible cultivars than the surgical blade procedure. With the surgical blade procedure, the discrimination between the resistant and intermediate cultivars was lower than with the razor blade procedure. It may be possible to increase severity and discrimination of the surgical blade procedure by increasing inoculum concentration. However, uniformity of CBB reaction on a given cultivar was greater with the surgical blade method, as evidenced by lower standard deviations calculated on ratings of individual plants within a genetically uniform cultivar. Applications also were more uniform between field workers when they used the surgical blade procedure.

The razor blade and surgical blade procedures are both excellent methods for the evaluation of resistance in bean to CBB. Both methods are easy to learn and utilize in the field when screening large populations; however, with the surgical blade it is possible to inoculate a larger number of plants in the same time period. Both procedures permit the evaluation in the field of other characters such as other diseases and architecture. This is an important factor for bean improvement programs that handle large populations.

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