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Coinoculation Effects of the Pathogens Causing Common Bacterial Blight, Rust, and Bean Common Mosaic in *Phaseolus vulgaris*

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Abstract. **Ten dry bean *(Phaseolus vulgaris* L.) cultivars/lines with differential reactions to rust were used in growth chamber experiments to determine rust *[Uromyces appendiculatus* (Pers.) Unger var. *appendiculutus, (U a)],* and common bacterial blight *Xanthomonas campestris* pv. *phaseoli* (E.F. Sm.) Dews. *(X c p)]* reactions on leaves when coinoculated with both pathogens. The *X c p-U a* necrosis symptoms were very different from those caused by *X c p* alone. Depending on the level of host susceptibility to rust, the *X c p* reaction remained confined within the rust pustule or spread beyond the pustule area, causing a necrosis of the entire leaf. Prior infection of bean seedlings with bean common mosaic virus (BCMV), NY-15 strain, reduced rust pustule size, but did not affect the reaction to *X c p.* Screening with *X c p* and BCMV can be done at the same time during the early vegetative stage, but the interactions of *U a* with *X c p* and of BCMV with *U a* need to be considered in screening for resistance.**

Rust, common bacterial blight *(X c p),* and bean common mosaic virus (BCMV) often occur together and affect dry bean production worldwide (Ballantine, 1978; Hampton, 1975; Yoshi, 1980). Resistances to *X c p,* rust, and BCMV need to be combined in cultivars for use in many bean production areas of the world.

The susceptibility of a plant to a pathogen is sometimes altered if the plant is already invaded by another pathogen (Carter, 1956; Dillon-Weston, 1927; Finke et al., 1986; Smith, 1951; Stall, 1955; Yarwood, 1950, 1969). The joint inoculation of *U a* and *X c p* was studied by Finke et al. (1986) and DeFaria and Hagedorn (1986) on separate trifoliolate leaves; no antagonistic or synergistic interaction was found between the two pathogens. Virus infection often increases host susceptibility to necrotrophic foliar fungal pathogens (Beniwal and Gudauskas, 1974; Bente, 1973). Studies on interactions between viruses and rusts of graminaceous hosts have shown that virus infection may decrease pustule density (Latch and Potter, 1977; Potter, 1982). However, some viruses have no effect on rust development. Raju et al. (1969) showed that development of *Puccinia recondita* sp. *tritici* was similar on virus-free and brome mosaic virus-infected wheat, but that rust developed more profusely on plants infected with wheat streak mosaic virus than on virus-free plants (Raju et al., 1969). We could not find reports of similar studies of rust development on dry bean in the presence or absence of a systemic virus. Hedges (1944, 1946a, 1946b) and Panzer and Nickeson (1959) studied the BCMV-bacterium effects on disease severity of *P. vulgaris* under field conditions. A synergistic effect of the two pathogens was noted only late in the growing season.

The objectives of this research were to determine the effect of prior inoculation of beans with the pathogens causing rust and BCMV on the reaction of the pathogens causing common blight and rust or common blight, respectively. This technique should be of importance to evaluate beans for multiple disease reaction, especially in tropical areas of the world where those diseases occur simultaneously. It can also be used in situations

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Table 1. Disease ratings of dry bean cultivars/lines inoculated singly and coinoculated with the pathogens causing rust (U a; Uromyces *appendiculatus* var. *appendiculatus)* and common blight *(X c p; Xanthomonas campestris* pv *phaseoli).*

Cultivars/lines	Rust index[z]		Bacterial-rust interaction rating[y]		Common blight rating[x]	
	Expt. 1 (RI_1)	Expt. 2 (RI_2)	Expt. 1 (BR_1)	Expt. 2 (BR_2)	Expt. 1 (CB_1)	Expt. 2 (CB_2)
CSW-643	0.8	0.1	3.3	2.8	3.5	2.9
KW-814	3.7	2.4	4.5	4.7	5.0	4.8
KW-780	4.2	---	5.0	---	5.0	---
KW-765	1.9	0.3	3.3	2.8	3.0	3.1
UI-114	3.8	3.2	4.8	4.7	5.0	5.0
Redlands Pioneer	3.3	---	4.8	---	4.3	---
Early Gallatin	3.9	1.2	5.0	5.0	5.0	4.5
US-3	4.9	1.7	5.0	4.8	5.0	5.0
Belneb	---	0.0	---	1.2	---	3.0
Harris	---	1.4	---	4.1	---	3.0

Simple correlation between RI_1 and RI_2 = +0.71*, BR_1 and BR_2 = +0.99*, and CB_1 and CB_2 = +0.95*.
Rank correlation between RI_1 and RI_2 = +0.54*, BR_1 and BR_2 = +0.97*, and CB_1 and CB_2 = +0.79*.
$LSD_{0.05}$ value to compare means for the same inoculation treatment for different cultivars/lines was 0.60 in both experiments.

[z]Rust index = Pustule grade × infection intensity.
[y]Bacterial-rust disease rating classes were: 1) no visible symptoms; 2) lustrous black flecks; 3) water-soaking and necrosis within the rust lesion; 4) water-soaking and necrosis surrounding rust lesion; 5) water-soaking and necrosis over the leaf surface.
[x]Common blight disease rating classes were: 1) no visible symptom; 2) small lesions on 1% to 5% of inoculated area; 3) lesions of moderate size on 6% to 25% of inoculated area; 4) lesions of varying size on 26% to 50% of inoculated area; 5) lesions of varying size on >50% of inoculated area.
*Significant at $P = 0.05$.

of limited space, or to obtain faster results of bean germplasm evaluation for resistance to more than one of the pathogens.

Materials and Methods

Coinoculation of U a and X c p. Bean plants were grown in a growth chamber (Expt. 1) to test the effect of the presence of *U a* on the "reaction of *X c p* in two experiments. A split-plot design was used with cultivars as main plots, replicated four times, and inoculation treatments as subplots. Eight bean cultivars, ranging from highly susceptible to moderately susceptible to a North Platte, Neb., *U a* isolate (US-NP-10-1), were used. Subplot treatments were as follows: 1) The abaxial surfaces of fully unfolded primary leaves of bean seedlings were inoculated with a water suspension of urediniospores (10^5 spores/ml) using a modified crown sprayer 7 to 12 days after seeding (Fisher Scientific Co.). The plants were incubated in a humidity chamber for 18 hr (overnight), then moved to a growth chamber at 20 to 25C. 2) The fully unfolded primary leaves were inoculated with five isolates of *X c p* from various geographic regions combined in a bacterial suspension (10^7 cfu/ml) sprayed to run off on the leaves. The five *X c p* isolates used were: CBP 184 (Colombia), DRL-827 (Dominican Republic), T-45 br (Puerto Rico), EK-11, and LB-2 (Nebraska). Differences in levels of virulence of these isolates have been reported (Zaiter et al., 1989). 3) The abaxial surfaces of fully unfolded primary leaves were inoculated with *U a* urediniospores as described previously. The leaves were then sprayed (2 weeks after this inoculation) to run off with *X c p* isolates when uredinia had erupted. No water soaking or wounding of the leaves occurred. Disease ratings for *U a* and *X c p* were made 10 days after inoculation with *X c p* (Table 1).

Six of the eight bean cultivars used in Expt. 1, along with 'Belneb-1' (rust-resistant) and 'Harris' (rust-susceptible) cultivars, were tested subsequently in Expt. 2 in a split-plot with six replicates (Table 1).

Table 2. Mean rust pustule diameter and disease reaction on dry bean cultivars/lines inoculated singly or coinoculated with the pathogens causing rust *(U a; Uromyces appendiculatus* var. *appendiculatus)* and bean common mosaic virus (BCMV).

Cultivars/lines	Type of plant[z]				
	Control		Infected		
	Expt. 1 (RVF_1)	Expt. 2 (RVF_2)	Expt. 1 (RVI_1)	Expt. 2 (RVI_2)	BCMV reaction[y]
	mm				
UI-111	0.58	0.55	0.50	0.48	S
U1-114	0.55	0.55	0.40	0.40	R
WM_1-85-39	NF	I	I	I	R
WM_1-85-43	NF	NF	NF	NF	S

Simple correlation between RVF_1 and RVF_2 = +0.99* and RVI_1 and RVI_2 = +0.99*.
$LSD_{0.05}$ values to compare means for the same inoculation treatment for different cultivars/lines in Expt. 1 = 0.04 and Expt. 2 = 0.06.
$LSD_{0.05}$ values to compare mean differences between RVF_1 and RVI_1 treatments = 0.02 and RVF_2 and RVI_2 treatments = 0.02.

[z]I = immune, no necrosis or other evidence of infection; NF = necrotic flecking without spores.
[y]R = resistant; S = susceptible.
*Significant at $P = 0.05$.

In both experiments, plants were grown in 15-cm (1.8-liter) clay pots (two per pot) containing 5 kg (1.4 liter) of steam-pasteurized potting medium (equal parts of sand, Sharpsburg silty clay loam soil, vermiculite, and peatmoss). Air was maintained ≈ 23C during 10/14 hr (light/dark) periods at a photosynthetic photon flux (PPF) of 340 μmol· s^{-1}·m^{-2} at a plant height 110 cm below the light source. High-pressure sodium and metal halide lamps were used.

Table 3. Common blight *(X c p)* reaction on leaves of dry bean cultivars/lines inoculated with *Xanthomonas campestris* pv. *phaseoli* on bean common mosaic (BCMV) control plants and on BCMV-inoculated plants.[z]

Cultivars/lines	BCMV-control	BCMV-infected	BCMV reaction
Sanilac	98	100	Susceptible
EP-1	39	43	Resistant
WM_1-85-57	40	53	Resistant
UI-114	100	100	Resistant

$LSD_{0.05}$ value to compare means for the same inoculation treatment for different cultivars/lines = 18.
$LSD_{0.05}$ value to compare means between different inoculation treatments = 8.

[z]*X c p* rating scale: 0% (no symptoms) to 100% necrosis of the leaf area inoculated with the multiple needle.

The pustule rating system described by Stavely (1984) was used (Table 2). Rust intensity (the percentage of leaf area with rust symptoms) was classified from 0% to 99% of the leaf area. A rust index, pustule grade x infection intensity, was used to evaluate the leaf rust disease reaction. The term "bacterial-rust necrosis" was used to describe the distinct bacterial/rust symptoms, and the ratings were based on the type of bacterial lesions surrounding the rust pustules (Table 1).

Coinoculation of U a *and BCMV.* Two separate experiments were conducted in growth chambers, replicated four times in each experiment. Bean plants were grown as in the *U a* and *X c p* coinoculation study.

The BCMV (NY-15 strain) was maintained in the dry bean cultivar Sanilac. Inoculum was prepared by picking leaves of 2- to 6-week-old BCMV-diseased plants, grinding them in a mortar and pestle, diluting 1:5 with distilled water, and mixing with 500-mesh Carborundum powder.

The following four cultivars/lines comprised the main plots arranged in a randomized complete block design; 'UI 111' and WM_1-85-43, both susceptible to BCMV (NY-15 strain), and 'UI-114' and WM_1-85-39, both resistant to the same BCMV strain. Subplot treatments were as follows: 1) The abaxial surface of fully unfolded primary leaves of seedlings were inoculated with rust urediniospores (US-NP-10-1); 2) One-half- to three-fourths-expanded primary leaves were rubbed lightly with the BCMV inoculum by hand; and 3) One-half- to three-fourths-expanded primary leaves were inoculated with BCMV and then the same primary leaves, when fully unfolded, were inoculated with rust urediniospores.

Disease ratings for virus and rust were made 14 days after the rust inoculation. The rust pustule rating system described by Stavely (1984) was used (Table 2).

Coinoculation of X c p *and BCMV.* Cultivars/lines comprised the main plots (four replications) in a randomized complete-block design with three inoculation treatments as subplots. The cultivars/lines included 'Sanilac' (susceptible to BCMV and X

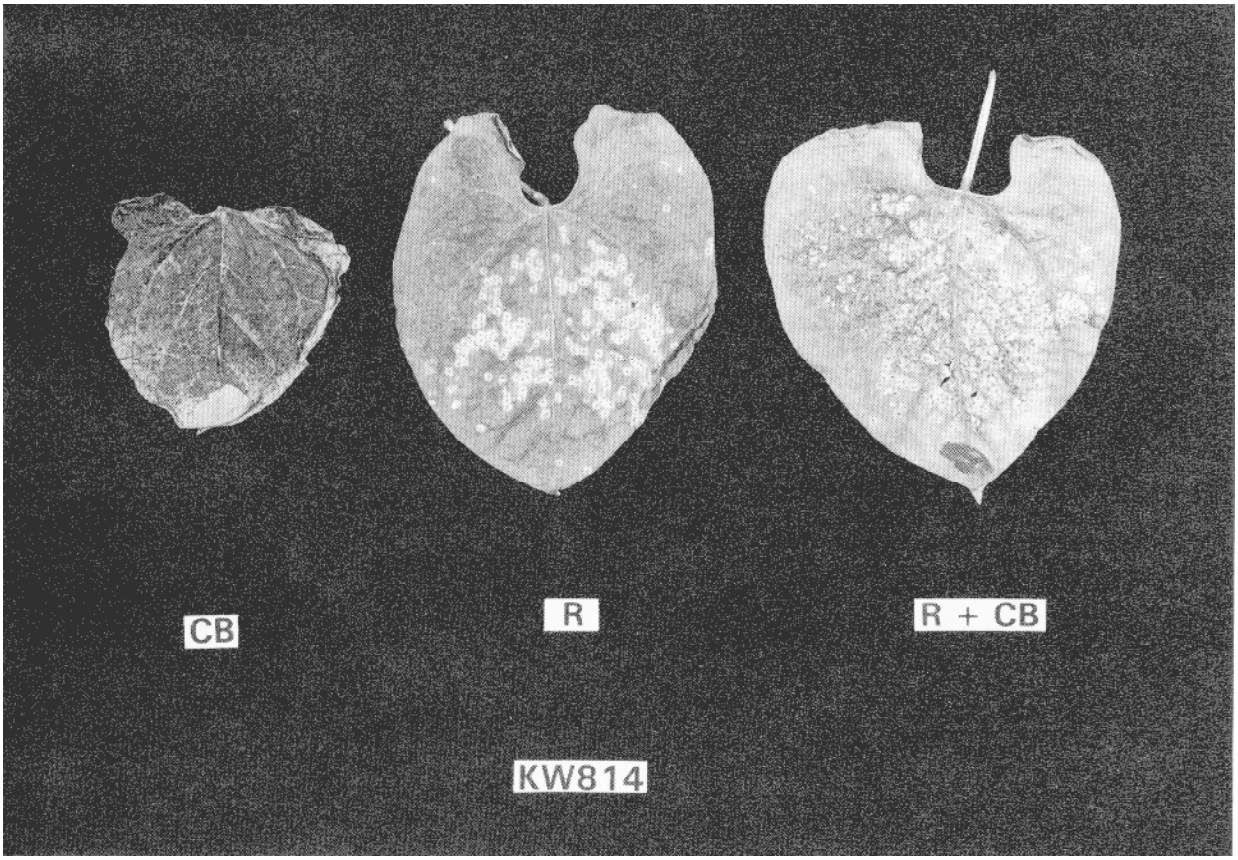

Fig. 1. Symptoms produced by single- and co-inoculation of *Xanthomonas campestris* pv. *phaseoli (X c p)* and *Uromyces appendiculatus* var. *appendiculatus (U a)* on fully expanded primary leaves of dry bean cultivar KW-814. **(left to right):** Common blight symptoms-inoculation with *X c p* alone; rust pustules—inoculation with *U a* alone; bacterial-rust necrosis symptoms following co-inoculation.

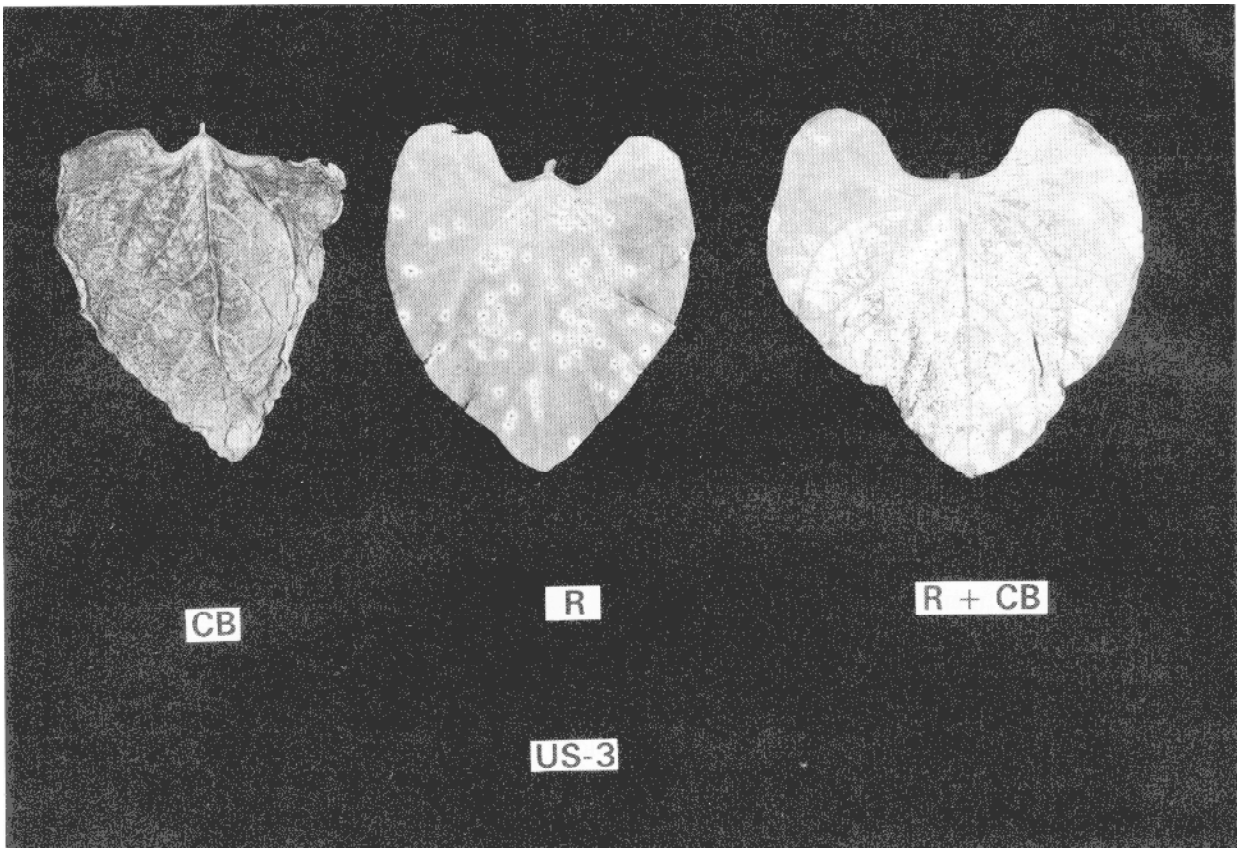

Fig. 2. Symptoms produced by single- and co-inoculation of *Xanthomonas campestris* pv *phaseoli (X c p)* and *Uromyces appendiculatus* var. *appendiculatus (U a)* on fully expanded primary leaves of dry bean cultivar US-3. **(left to right):** Common blight symptoms—inoculation with *X c p* alone; rust pustules—inoculation with *U a* alone; bacterial-rust necrosis symptoms following co-inoculation.

c p), EP-1 (resistant to BCMV and X c *p),* 'UI 114' (resistant to BCMV and susceptible to *X c p),* and WM_1-85-57 (resistant to BCMV and *Xcp).* Inoculation methods for the two pathogens were those described previously. Bean plants were grown as in the *U a* and X c *p* coinoculation study.

The three inoculation treatments were as follows: 1) Fully unfolded trifoliolate leaves of seedlings were inoculated with X c *p;* 2) One-half- to three-fourths-expanded primary leaves were inoculated with BCMV; and 3) One-half- to three-fourths-expanded primary leaves of seedlings were inoculated with BCMV (NY-15) and later the first trifoliolate leaves were inoculated with X c p.

Disease ratings for common blight and BCMV were made 12 days after inoculation (Table 3).

Results and Discussion

Coinoculation of U a *and* X c p. Rust accentuated the damage caused to foliage by *X c p* (Figs. 1 and 2). We suggest that when uredinia erupted, bacteria entered the wounded tissue and caused the necrotic lesions on the leaves. Depending on the level of host susceptibility, the bacterial lesions were confined within the rust lesions or extended over the entire leaf (Figs. 1 and 2). These bacterial-rust necrosis symptoms were very different from those caused by *X c p* alone. The correlations between rust index and lesion size in bacterial-rust necrosis interactions were +0.93 and +0.78 in Expts. 1 and 2, respectively. Susceptibility to bacterial-rust necrosis was associated with the rust index and with susceptibility to *X c p* (Table 1). The correlations between results of all inoculation treatments in Expts. 1 and 2 were high, indicating repeatability (Table 1). The interaction of the *U a* and *X c p* would cause plant stress at least as great as either pathogen alone and could be additive on beans. This situation can arise in the tropics where plants are often first infected with *U a* and then later with X c *p.*

Coinoculation of U a and BCMV. Rust development was similar on control and virus-infected plants irrespective of the leaf surface inoculated (Table 2). Flecking occurred 8 days after 'inoculation. White pustules formed after 12 days and erupted 2 days later in both experiments. The correlations between the two experiments under BCMV-free and BCMV-infected plants were very high (Table 2).

Rust pustule size was smaller in BCMV-infected leaves than in control plants of 'UI-111' and 'UI-114', but there was no difference in the type of rust reaction for WM_1-85-43 and WM_1-85-39 in both experiments (Table 2). 'UI-114' and 'UI-111' remained rust-susceptible, and WM1-85-43 and WM_1-85-39 remained rust-resistant in the presence of BCMV. Pustule intensity and spore production need to be studied in the future in healthy vs. virus-infected leaves because infection of faba bean *(Vicia Faba* L.) with bean yellow mosaic virus (BYMV) reduced rust pustule density of *Uromyces viciae-fabae* (Omar et al., 1986).

Because prior infection of dry beans by BCMV decreased host susceptibility to subsequent rust infection, but did not change a cultivar ranking from resistant to susceptible, or vice versa, this pathogen interaction should be considered in breeding pro-

warns with the obiective of developing resistance to both pathogens. Multiple inoculation is therefore feasible and reliable, and can be used in screening for resistance, provided that control cultivars for each of the diseases are included in the tests.

Coinoculation of X c p *and BCMV.* There was no difference in symptoms induced by each pathogen when inoculated singly or co-inoculated on each of the four cultivars/lines (Table 3). Thus, presence of one pathogen did not affect the reaction to the other pathogen.

These results confirm those of Panzer and Nickeson (1959), where no synergistic or antagonistic effects were found at the early vegetative stage of beans in the field. However, they noted a synergistic effect of the two pathogens late in the growing season. The latter interaction, associated with maturity, was not investigated in this study.

Coinoculation with *X c p* and BCMV in the seedling stage gave the same results as inoculating separately, and thus coinoculation can be effectively used by breeders in selecting for reaction to both pathogens.

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