

## Insect Transmission of Pathogenic Xanthomonads to Bean and Cowpea in Puerto Rico

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### ABSTRACT

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Bacterial blight lesions on beans (*Phaseolus vulgaris*) and cowpeas (*Vigna unguiculata*) frequently were associated with insect-feeding injuries. Three pathogenic xanthomonads, *Xanthomonas phaseoli* (Xp), *X. phaseoli* var. *fuscans* (Xpf), and *X. phaseoli* f. sp. *vignicola* (Xpv) (all members of the *X. campestris* group) were isolated from the washings of five leaf-feeding insect species, *Ceratomyza ruficornis*, *Chalcidoderma ebeninus*, *Diaprepes abbreviata*, *Empoasca* sp., and *Nezara viridula* collected from bacterial blight-infected bean plantings at Isabela, Puerto Rico. Pathogenicity tests with 51 pathogenic isolates from these insects indicated that 16 were either Xp or Xpf and 35 were Xpv. In controlled feeding trials, infection of caged bean and cowpea plants by pathogenic xanthomonads took place only

at the feeding sites of naturally-infested *C. ruficornis* and *D. abbreviata*. Infection also resulted when four of the insect species (*C. ebeninus* was not tested) were artificially infested with different bacterial isolates. However, no infection occurred after 48 hr when *C. ruficornis* and *D. abbreviata* were infested and transferred at daily intervals to healthy test plants. Feeding injuries caused by Xpv-infested or noninfested *D. abbreviata* on noninoculated or inoculated bean leaves, respectively, greatly enhanced lesion development. Bean blight bacteria were isolated from fresh feces of *C. ruficornis* and *D. abbreviata* that had fed on infected bean leaves. Several xanthomonads survived for periods up to 19 days on the bodies of live and dead *C. ruficornis* and *D. abbreviata*.

*Additional key words:* food legumes, tropics, vectors, strains.

In Puerto Rico, beans (*Phaseolus vulgaris* L.) and cowpeas [*Vigna unguiculata* (L.) Walp.] usually are grown during the rainy season which extends from May to November. During this period of high rainfall, high humidity, and warm temperatures bacterial blight of bean and cowpea caused by *Xanthomonas phaseoli* (E. F. Sm.) Dows. and *X. phaseoli* f. sp. *vignicola* (Burkh.) Sabet, respectively, often are important diseases (10). Although these bacteria now are grouped under *X. campestris* (2), in this paper they will be considered as distinct. Insect injury to bean and cowpea foliage generally is prevalent during the rainy period, although it is not as extensive as during the dry season.

During the rainy season in western Puerto Rico, in the vicinity of Isabela, bacterial bean blight occasionally appeared to be associated with insect-feeding injuries in experimental bean plantings (Fig. 1). Others (4, 7, 11) have suggested that insects are disseminators of bean

bacterial blight pathogens, but little evidence was presented. These studies were initiated to determine whether insects were responsible in part for the dissemination and transmission of bacterial blight pathogens of bean and cowpea in Puerto Rico.

In this paper, dissemination refers to the transfer of bacterial inocula by insects from diseased to healthy plants. Transmission includes dissemination and inoculation by insects during feeding which results in infection and disease development in a susceptible host.

### MATERIALS AND METHODS

Both healthy and bacterial blight-infected bean leaves with insect injuries were collected from experimental plantings at the U.S. Department of Agriculture Field Station and the Puerto Rican Department of Agriculture Substation at Isabela, Puerto Rico during July-November, 1973. Isolations were made on nutrient agar (NA) or yeast extract-dextrose-calcium carbonate agar (YDCA) (8) from: (i) bacterial blight lesions associated with insect feeding injuries, (ii) blight lesions not

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associated with insect damage, and (iii) insect feeding injuries without bacterial blight symptoms.

Test plants used in the inoculation studies were the bean cultivars Bountiful and La Vega and the cowpea cultivars PR-V-70-10-R65 and Early Ramshorn. These bean and cowpea cultivars are very susceptible to two *Xanthomonas* strains present in Puerto Rico (10). The test plants were seeded in steam-sterilized soil in perforated, 14 × 16 cm black plastic bags. Plants in the primary leaf stage (8-10 days old) were used in all insect feeding studies. Test plants in the primary or first trifoliolate leaf stage were used to verify the pathogenicity of bacterial isolates.

Inoculation with bacterial isolates or wash water from insects was done by applying the inoculum suspension to Carborundum-dusted leaves with the thumb and forefinger or by forcing a sterile needle into the stem through a drop of inoculum placed in a petiole axis at a node. A known virulent bacterial isolate was included as a check in all pathogenicity tests. After inoculation, test plants were placed in a plastic mist chamber (incubator) (10) for periods up to 4 days. Temperatures in the incubator ranged from 19-35 C, with a mean of 28 C. Reisolations were made from inoculated plants by placing small pieces of tissue from the advancing edge of lesions or cankers in sterile distilled water for 15-20 min and then streaking a loopful of this liquid onto the surface of NA or YDCA.

Bacterial isolates were grown on NA or YDCA. Cultures were incubated at room temperature (22-24 C). Turbid aqueous suspensions ( $10^9$  cells/ml) from 2- to 4-day-old cultures were used in all inoculation studies. Bacterial isolates were stored in distilled water at 4-6 C.

Insects used in various experiments were collected with an aspirator, a net, or by hand, and then placed in paper bags. After transporting them to the laboratory, some insects were used immediately in feeding trials, but others

were placed on healthy bean or cowpea plants in screen cages for at least 3 days before they were used in different transmission studies. Insects collected in the field also were placed individually in vials containing 2-5 ml of sterile distilled water. Later, in the laboratory, a loopful of wash water from each vial was streaked on NA or YDCA, and the remaining liquid was used to inoculate the leaves and nodes of bean and cowpea test plants.

The following insects were collected from bean plantings at Isabela, Puerto Rico, and tested as possible vectors of bacteria pathogenic to bean and cowpea:

Coleoptera: *Cerotoma ruficornis* Oliv., Chrysomelidae; *Diaprepes abbreviata* L., Curculionidae; and *Chalcodermus ebininus* Boheman, Curculionidae.

Hemiptera: *Nezara viridula* L., Pentatomidae; and *Empoasca* sp., Cicadellidae.

In feeding trials, insects were placed in round, clear plastic cylinders that fitted snugly around the black plastic bags containing the test plants. The top of each cylinder was covered with cheesecloth. Insects were inserted through a hole in the cheesecloth which then was plugged with cotton.

Cultures of *Xanthomonas* spp. used in these studies were *X. phaseoli*, ATCC 9563; *X. phaseoli* var. *fuscum* (Burkh.) Starr & Burkh., ATCC 13464; and *X. phaseoli* sp. *vignicola*, ATCC 11648 [syn. *X. vignicola* Burkh. (designated here as isolates Xp 9563, Xpf 13464, and Xp 11648, respectively). Bacterial cultures that were used previously by the authors (10) and included in this study were Xp 253 and Xpv 113. In pathogenicity tests, Xp 253 was similar to ATCC Xp 9563 (infected bean only), but Xpv 113 was similar to ATCC Xpv 11648 (infected both bean and cowpea).

The effects of *D. abbreviata* feeding injuries to bean

leaves, in relation to the incidence of bacterial blight, were studied. The plants were: (i) nondamaged, noninfested; (ii) noninfested, noninfested with a bacterial suspension; (iii) noninfested, noninfested with a bacterial suspension and exposed to noninfested beetles; (iv) noninfested, noninfested with bacteria; (v) exposed to a bacterial suspension and beetles only. Water on bean leaves with treatment was terminated in an incubator for 4 days.

To study the survival of *C. ruficornis* on sterile petri dishes with bacterial isolates Xpv 113, naturally by the bacteria, periods of 18-24 hr, then sterile petri dishes, intervals up to 6 h containing 2 ml sterile suspension was streaked on the nodes of healthy plants from insects fed on controls.

Survival of *Xanthomonas* and dead (autoclaved) *C. ruficornis*. Living and dipping them into cells/ml of different insects were placed on infested autoclaved in dishes at room temperature one to three insects distilled water. After was inoculated into plants.

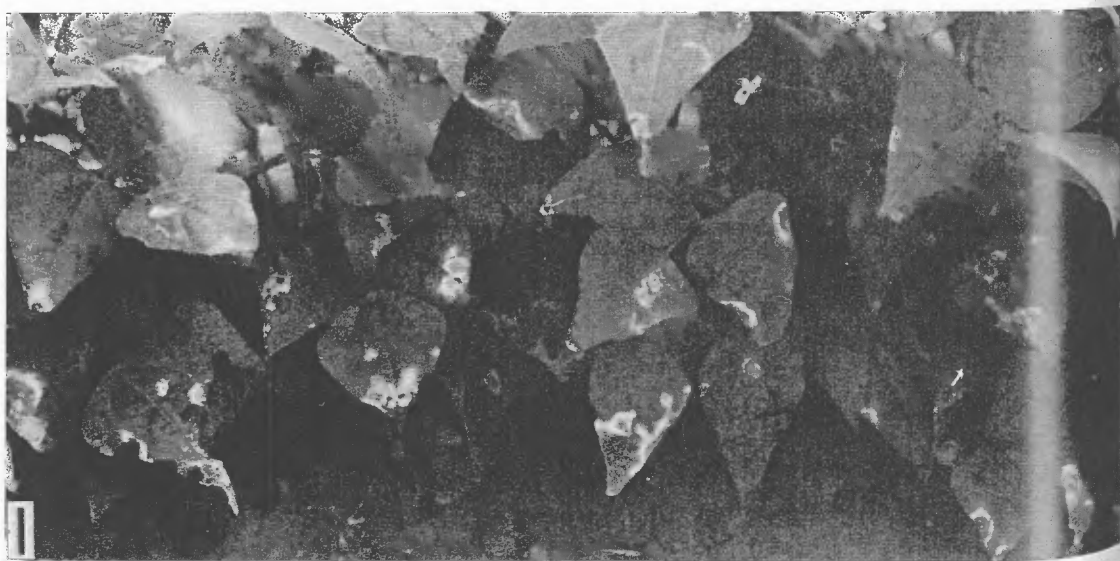


Fig. 1. Bean plants naturally-infected with bacterial blight at Isabela, Puerto Rico. Some blight lesions are associated with insect feeding injuries which occur mainly at the edges of leaves.

Isolation of pathogenic bacteria from naturally infected bean insects. Most insect damage to cowpea appeared to be caused by beetles. *C. ruficornis* populations of *C. ruficornis* those of *D. abbreviata* damage to the foliage of these two insects are generally confined to the outer edge of the leaf lamina (Fig. 2), while the feeding sites of *Empoasca* sp. and *N. viridula* are generally associated with bacterial blight lesions. The lesions subsequently to expansion.

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leaves, in relation to methods and time of inoculation, on the incidence of bacterial blight lesions were investigated. The plants were: (i) sprayed with distilled water (non-damaged, noninoculated control), (ii) sprayed with a bacterial suspension and incubated for 24 hr, (iii) sprayed with a bacterial suspension, incubated for 1 hr and then exposed to noninfested beetles, (iv) exposed to noninfested beetles for 24 hr, followed by inoculation with bacteria, (v) exposed to beetles infested by dipping in a bacterial suspension, and (vi) exposed to noninfested beetles only. Water or bacterial suspensions were sprayed on bean leaves with an aerosol spray kit. When each treatment was terminated, plants were placed in an incubator for 4 days.

To study the survival of *Xanthomonas* bacteria in insect feces, *C. ruficornis* and *D. abbreviata* contained in sterile petri dishes were fed bean leaves infected with either isolates Xpv 113, Xpf 13464, or bean leaves infected naturally by the bacterial blight pathogen. After feeding periods of 18-24 hr, the insects were transferred to empty sterile petri dishes. Feces were collected at periodic intervals up to 6 hr after transfer, placed in vials containing 2 ml sterile distilled water and shaken. The suspension was streaked on NA and also inoculated into the nodes of healthy bean and cowpea test plants. Feces from insects fed on healthy bean leaves were used as controls.

Survival of *Xanthomonas* isolates was tested on live and dead (autoclaved) adults of *D. abbreviata* and *C. ruficornis*. Living and autoclaved insects were infested by dipping them into turbid bacterial suspensions ( $10^7$  cells/ml) of different bacterial isolates. Infested living insects were placed on bean and cowpea test plants, and infested autoclaved insects were incubated in sterile petri dishes at room temperature. At different time intervals, one to three insects were washed with 2 ml of sterile distilled water. After streaking on NA, the wash water was inoculated into the nodes of bean and cowpea test plants.

## RESULTS

**Isolation of pathogenic *Xanthomonas* bacteria from naturally infected bean leaves damaged by leaf-feeding insects.**—Most insect-feeding damage to bean and cowpea appeared to be caused predominantly by two beetles, *C. ruficornis* and *D. abbreviata*. In the field, populations of *C. ruficornis* increased more rapidly than those of *D. abbreviata* and generally inflicted greater damage to the foliage of both crops. The feeding habits of these two insects also differed markedly. *Cerotoma* generally confined its feeding to the center portions of the lamina (Fig. 2), while *Diaprepes* fed mainly along the outer edge of the leaf (Fig. 3). It was difficult to identify the feeding sites of the leaf-sucking insects, including *Empoasca* sp. and *N. viridula*.

Bacterial blight lesions on bean leaves were associated with feeding injuries caused by *C. ruficornis* and *D. abbreviata*. The lesions appeared to originate at and subsequently to expand from the damaged areas (Fig. 2, 3).

*Xanthomonas* spp. cultures isolated from blight-infected feeding injuries were similar in colony

morphology, physiological properties, and pathogenicity to pathogenic xanthomonads isolated from lesions on bean leaves not associated with insect injuries and to bacteria previously isolated in Puerto Rico (10). Occasionally, yellow-pigmented bacteria with colony morphology similar to *Xanthomonas* spp. were isolated from insect feeding sites not associated with bacterial blight symptoms; these bacteria, however, were not pathogenic to bean or cowpea.

**Isolation of pathogenic xanthomonads from insects collected from blighted bean plantings.**—Xanthomonads pathogenic to bean and cowpea were isolated from five insect species collected from bean plants infected with bacterial blight (Table 1). Insects that carried pathogenic bacteria were of the same species that caused damage to the foliage of bean and cowpea plants. These were the leaf-chewing Coleoptera *C. ruficornis*, *C. ebeninus*, and *D. abbreviata*; and the leaf-sucking Hemiptera *Empoasca* sp. and *N. viridula*. The percentage of individuals that carried pathogenic xanthomonads varied from 11 to 86 (Table 1). Pathogenic bacteria were isolated more frequently from *C. ruficornis* than from the other species tested.

In pathogenicity tests with 127 xanthomonad-like isolates, less than half were pathogenic to bean and cowpea (Table 1). The pathogenic isolates were of two types, one which infected bean only (Xp and Xpf) and the other which infected both bean and cowpea (Xpv).

**Transmission of blight bacteria to bean and cowpea by naturally and artificially-infested foliage-feeding insects.**—Several leaf-feeding insects collected in bean fields from plants infected with bacterial blight were tested for ability to transmit the pathogen to healthy bean and cowpea test plants. Two naturally-infested beetles, *C. ruficornis* and *D. abbreviata*, transmitted bacteria that induced bacterial blight in bean and cowpea. The blight

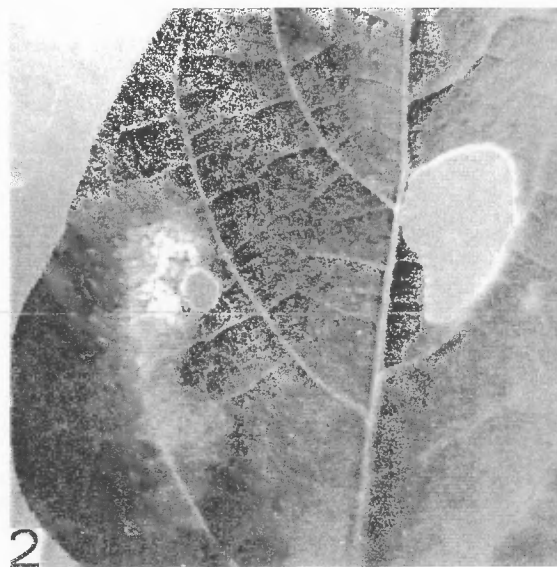


Fig. 2. A bean leaf fed upon by *Cerotoma ruficornis* that had been artificially-infested with an isolate of *Xanthomonas phaseoli* var. *fuscans*. One feeding site (at the left) is infected.

lesions that developed at insect-feeding sites on bean test plants were identical to those observed on naturally-infected, insect-damaged bean foliage. The bacterial blight lesions originated only at feeding sites. Reisolation from these lesions yielded pathogenic xanthomonads. Less than 10% of *C. ruficornis* and *D. abbreviata* found to be disseminating pathogenic xanthomonads could transmit bean blight bacteria.

Single, artificially-infested adults of *C. ruficornis* and

*D. abbreviata* transmitted isolate Xpv 113 to bean test plants in controlled feeding tests (Table 2). Three of 12 insects did not feed on the leaves of the bean test plants during the 24-hr test feeding period. One or more lesions formed at some of the feeding sites of all but one feeding insect. Although *D. abbreviata* caused the greatest damage to the bean leaves, approximately 30% of the feeding sites of each insect species were infected.

Although pathogenic *Xanthomonas* bacteria could

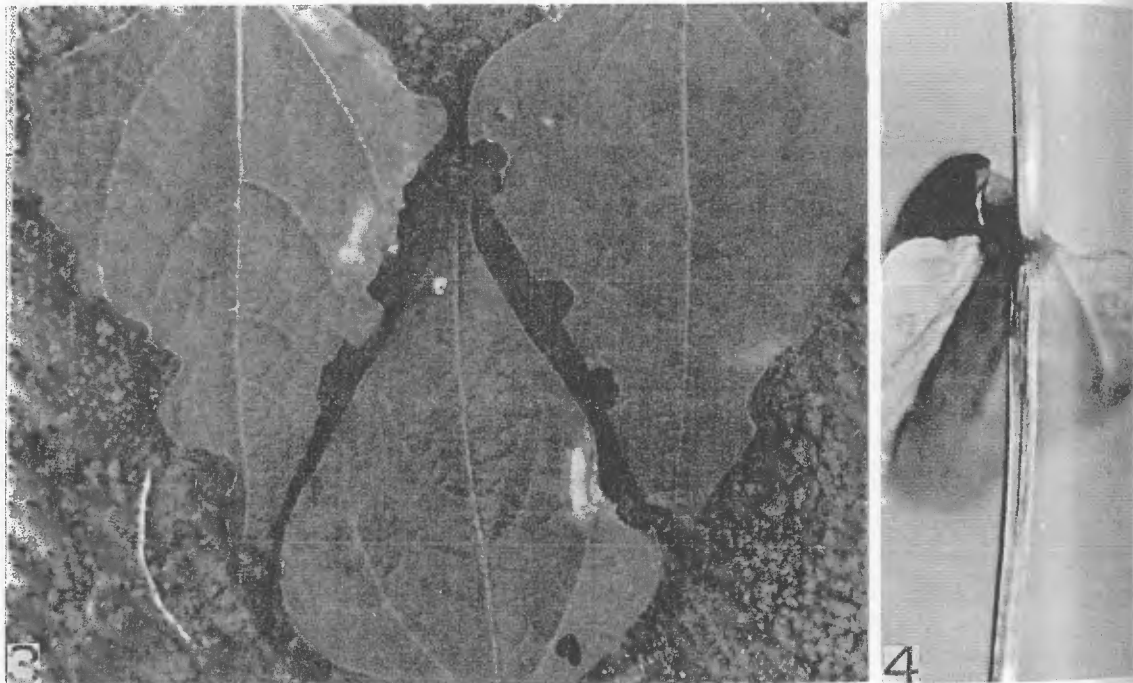


Fig. 3-4. 3) Leaves of bean plants naturally-infected with bacterial blight at Isabela, Puerto Rico. Most lesions (light areas) are found to be associated with feeding injuries caused by *Diaprepes abbreviata*. 4) A canker that developed on the stem of a cowpea plant that was fed upon by *Nezara viridula* infested with an isolate of *Xanthomonas phaseoli* f. sp. *vignicola*.

TABLE 1. Insect species collected from bean plants infected with bacterial blight and the pathogenicity of xanthomonads isolated from these insects to bean and cowpea<sup>a</sup>

Insect species	Insects tested (no.)	Insects carrying pathogenic xanthomonads (no.) <sup>c</sup>	No. isolates pathogenic to:	
			Bean <sup>b</sup>	Bean + Cowpea
<i>Cerotoma ruficornis</i>	22	19	5	14
<i>Chalcoedermus ebeninus</i>	5	4	1	3
<i>Diaprepes abbreviata</i>	37	11	3	8
<i>Empoasca</i> sp.	53	12	6	6
<i>Nezara viridula</i>	10	5	1	4
Total	127	51	16	35

<sup>a</sup>Pathogenicity determined by inoculating the leaves or nodes of La Vega or Bountiful bean, and of Early Ramshorn or PR-V-70-R65 cowpea.

<sup>b</sup>Isolates pathogenic to bean only.

<sup>c</sup>Isolates pathogenic to both bean and cowpea.

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TABLE 2. primary leaf *Cerotoma ru*

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TABLE 3. *Diaprepes a*

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isolated from wash water of *Empoasca* sp. and *N. viridula* (Table 1), no transmission resulted when these insects (collected from blight-infected bean plants) were transferred directly to caged test plants. However, transmission did occur when field-collected insects were infested with a suspension of isolate Xpv 113. Only a few lesions developed on bean leaves that had been fed upon by infested *Empoasca* sp. The lesions were observed to originate only at feeding sites. Infested *N. viridula* fed primarily on the stems of test plants. No bacterial lesions developed on the leaves, but large cankers formed on the stems of several cowpea test plants (Fig. 4). Plants with

cankers wilted and eventually died. Transmission tests were not carried out with *C. ebeninus* due to the difficulty in obtaining weevils although they were shown to be disseminators of pathogenic xanthomonads (Table 1).

**Serial transmission of xanthomonads by infested beetles.**—Both *C. ruficornis* and *D. abbreviata* transmitted pathogenic bacteria from naturally-infected bean leaves to bean test plants within the first 24-hr feeding period, but not on subsequent daily transfers (Table 3). Artificially-infested *D. abbreviata* transmitted bacterial isolates Xp 253, Xpf 13464, and Xpv 113 during the first 48 hr (two transfers), whereas *C. ruficornis* transmitted only isolate Xpv 113 for a similar period. Lesions were more numerous at feeding sites of *D. abbreviata* infested with the three bacterial isolates than in those of similarly infested *C. ruficornis* (Table 3). Bacterial lesions did not develop at the feeding sites of either species after 48 hr, although pathogenic bacteria could be isolated from washings of the insects.

**Correlation of insect feeding-damage with incidence of bacterial blight lesions.**—The feeding activity of *D. abbreviata*, as determined by the number of feeding sites per leaf, was similar in treatments c to f (Table 4). Insect injuries influenced markedly the incidence of bacterial lesions developing on bean leaves in the inoculated series (treatments b to e). The number of bacterial lesions per leaf in treatment c (noninfested insects fed shortly after inoculation) and treatment e (infested insects only) were greater by factors of 6.6 and 4.8, respectively, than treatment b (nondamaged, inoculated control) (Table 4). The noninoculated, nondamaged, and damaged controls (treatments a and f) remained healthy in all trials.

The number of blight lesions per leaf followed a Poisson distribution. The chi-squares for treatments b, c, d, and e were not significant ( $P = 0.05$ ). The probability ( $p$ ) of obtaining one or more lesions per leaf in treatment b (nondamaged, inoculated control) was 0.59, but  $p$  values for treatments c and e were 0.99 and 0.98, respectively (Table 4). There was a good correlation between the

TABLE 2. Transmission of pathogenic bean blight bacteria to primary leaves of bean by single artificially-infested adults of *Ceratomyza ruficornis* and *Diaprepes abbreviata*

Insect no.	Insect species <sup>a</sup>			
	<i>C. ruficornis</i> <sup>b</sup>		<i>D. abbreviata</i> <sup>b</sup>	
	Feeding sites (no.)	Lesions (no.)	Feeding sites (no.)	Lesions (no.)
1	3	2	8	2
2	7	2	38	13
3	3	0	7	4
4	12	3	13	3
5	0	0	6	1
6	0	0	0	0
Total	25	7	72	23

<sup>a</sup>Insects were infested with bacteria when they walked on the surface of nutrient agar seeded with *Xanthomonas phaseoli* f. sp. *vignicola* isolate 113.

<sup>b</sup>Infested insects were given a 27-hr test feeding period on the primary leaves of three healthy La Vega or Bountiful bean seedlings before the test plants were placed in a plastic mist chamber for 72 hr.

TABLE 3. Transmission of pathogenic *Xanthomonas* spp. bacteria to primary leaves of Bountiful bean by *Ceratomyza ruficornis* and *Diaprepes abbreviata* at various times after artificial infestation of the insects

Insect species	Bacterial source	Lesions per 100 feeding sites			
		Insects transferred at day:			
		1	2	3	4
<i>C. ruficornis</i> <sup>a</sup>	Infected Leaf <sup>b</sup>	11.9	0	0	0
	Xpv 113 <sup>c</sup>	13.1	0.6	0	0
	Xp 253	8.6	0	0	0
	Xpf 13464	1.4	0	0	0
<i>D. abbreviata</i>	Infected Leaf	3.8	0	0	0
	Xpv 113	30.6	5.6	0	0
	Xp 253	38.1	5.6	0	0
	Xpf 13464	12.6	1.6	0	0

<sup>a</sup>An average of 10 adult insects were given a 20- to 24-hr test feeding period on 7-10 Bountiful or La Vega bean seedlings. Insects were transferred to healthy plants at daily intervals without access to a fresh source of bacterial inoculum. Subsequent to the test feeding period, plants were placed in a plastic mist chamber for 72 hr.

<sup>b</sup>Insects were fed for 24 hr on naturally infected bean leaves from Isabela, Puerto Rico.

<sup>c</sup>Insects were placed in a turbid suspension ( $10^9$  cells/ml) of different bacterial isolates for 50-80 min. The isolates were: Xpv 113, local isolate of *X. phaseoli* f. sp. *vignicola*; Xp 253, local isolate of *X. phaseoli*; and Xpf 13464, American Type Culture Collection isolate 13464 of *X. phaseoli* var. *fuscans*.

numbers of bacterial lesions per leaf and feeding injuries per leaf (treatments c and e).

**Survival of *Xanthomonas* bacteria in feces and on bodies of *Cerotoma ruficornis* and *Diaprepes abbreviata*.**—Isolates Xpv 113, Xpf 13464, and pathogenic *Xanthomonas* spp. cultures from naturally-infested bean leaves remained viable and retained their pathogenicity to bean and cowpea after passing through the alimentary canal of *C. ruficornis* and *D. abbreviata*. Viable, pathogenic bacteria (generally  $<10^3$  colonies/ml) were isolated from the feces of both insect species up to 6 hr after removal of the insects from infested bean leaves. Although fecal droppings were collected at frequent intervals, it is possible that prior to removal some could have become contaminated with pathogenic bacteria through contact with infested insects or with bacteria adhering to the surface of the petri dish. No pathogenic bacteria were isolated from feces of control insects.

Isolate Xpv 113 survived on living, infested *D. abbreviata* and *C. ruficornis* for 11 and 19 days, respectively, whereas isolate Xpf 13464 remained viable for 10 days on both species. Isolates Xp 9563, Xpf 13464, Xpv 113, and Xpv 11648 survived on sterilized, dead adults of *D. abbreviata* for 12, 12, 14, and 12 days, respectively. The number of pathogenic bacterial colonies per milliliter of wash water from dead insects infested with the different *Xanthomonas* isolates was high ( $10^5$  to  $10^6$ ) at 12-14 days when the tests were discontinued. All bacterial isolates tested at the end of the sampling period retained pathogenicity to bean and cowpea.

#### DISCUSSION

The objective of this investigation was to establish the role of different foliar-feeding insects in the dissemination and transmission of pathogenic xanthomonads to bean and cowpea under field conditions in Puerto Rico. Extended field observations had indicated the possibility, and our data had confirmed, that different insect species

might be vectors of bean blight bacteria. Naturally or artificially infested insects were used to demonstrate a vector relationship with pathogenic bean blight bacteria in controlled feeding experiments. It should be noted, however, that not all naturally or artificially infested insects were vectors of bean blight bacteria. Ideally, we should have used only naturally infested insects in these studies, but this was not possible because bean blight and different insect species occurred seasonally and collecting and maintaining sufficient naturally infested insects to carry out various transmission and survival experiments proved difficult.

In studies with 67 pathogenic Puerto Rican *Xanthomonas* spp. isolates from bean, Vakili et al. (10) found that 84% of the isolates were of the Xpv strain (pathogenic to both bean and cowpea). Our data (Table 4) also showed a similar predominance of Xpv in the strains being disseminated by naturally infested, foliar-feeding insects. Pathogenicity to bean and cowpea was the only reliable criterion that could be used to differentiate Xpv from Xp or Xpf cultures.

The efficiency with which different insect species transmitted *Xanthomonas* spp. pathogens to bean varied considerably. Insect transmission of the SFR strain of *Pseudomonas solanacearum* to banana (3) suggests that success of insects as vectors of bacterial plant pathogens depends upon (i) insect numbers and activity, (ii) abundance, viability, and virulence of bacterial inoculum, and (iii) the frequency and susceptibility of infection sites. In this study, feeding behavior appeared to affect the insect's ability to transmit bacterial pathogens. For example, leaf-chewing species were more effective in transmitting bean blight bacteria to bean and cowpea than were leaf-sucking types. Because more leaf cells may be damaged by the former while feeding, additional sites for infection are available to pathogenic bacteria.

In blight-infested bean plants, foliar feeding insects are likely to be contaminated with pathogenic bacteria by feeding upon or crawling over infected tissues. The

TABLE 4. Effects of feeding injuries to the primary leaves of bean seedlings by artificially infested *Diaprepes abbreviata* on the incidence of bacterial blight lesions induced by *Xanthomonas phaseoli* f. sp. *vignicola* isolate 113

Treatment <sup>1</sup>	Leaves <sup>a</sup> (no.)	Lesions/leaf (avg. no.)	Feeding sites/leaf (avg. no.)	$\chi^2$	p
a) Water control	37	0	0	...	...
b) Bacteria only	38	0.9	0	2.8	0.59
c) Bacteria + insects	40	5.9	9.8	7.8	0.99
d) Insects + bacteria	41	1.2	12.0	3.8	0.65
e) Infested insects	39	4.3	10.5	7.8	0.98
f) Noninfested insects	35	0	10.4	...	...

<sup>1</sup>Descriptions of treatments a to f:

- a = Plants sprayed with sterile distilled water (nondamaged, noninoculated control). At the termination of treatments plants were placed in a plastic mist chamber for 4 days.  
 b = Plants sprayed with a bacterial suspension and placed in a mist chamber 24 hr after inoculation (nondamaged, inoculated control).  
 c = One hr after spraying leaves with a bacterial suspension, 10 noninfested insects were placed on plants for 24 hr.  
 d = Ten noninfested insects fed on plants for 24 hr and the leaves then were inoculated with bacteria.  
 e = Ten insects were infested by dipping them in a bacterial suspension and placing them in contact with test plants for 24 hr.  
 f = Plants fed upon by 10 noninfested insects for 24 hr (noninfested, insect control).

<sup>a</sup>Results of two experiments with 9-11 plants per experiment.

bacterial inoculum naturally infested regurgitated fluids, levels through desiccation. Apparently active cells of the surface of plant tissue artificially infested xanthomonads 24-48 hr. Adverse effects of pathogenic bacteria on insects after 4 days, reduction of viable cells, and an attenuation in viability.

The role of epiphytic bacterial bean blight is poorly understood. Xpv may exist as an apparently healthy bacterial pathogen and warm temperatures where these climatic conditions contribute to a high survival of these bean blight bacteria also on foliar-feeding insects on bean plantings. Wound infection remains unknown.

The effect of insect transport of bacterial pathogens among plantings of bean is unknown. Strong evidence of spread of bacteria at localities. Plant pathogens may survive extended periods on insects or contaminated state (6). Whether this is a source of primary infection.

Of the two naturally occurring flies, whereas *C. ruficornis*

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bacterial inoculum carried on the exoskeleton of naturally infested insects or present in their feces or regurgitated fluids, probably is reduced quickly to low levels through desiccation or exposure to ultraviolet irradiation. Apparently, a similar high mortality occurs with active cells of other bacterial plant pathogens on the surface of plant tissues (5, 6, 9). Therefore, the failure of artificially infested insects to transmit pathogenic xanthomonads 24-48 hr after infestation may result from adverse effects of the environment. In cases where pathogenic bacteria were isolated from washings of these insects after 4 days, the lack of infectivity may be due to a reduction of viable cells below an infection threshold or to an attenuation in virulence of these cells.

The role of epiphytes (5) in the epidemiology of bacterial bean blights under natural field conditions is poorly understood. In Puerto Rico, isolates Xp, Xpf, and Xpv may exist as resident epiphytes on the foliage of apparently healthy bean plants. Epiphytic growth of bacterial pathogens generally is favored by high humidity and warm temperatures (5, 6). In areas like Puerto Rico where these climatic conditions prevail, epiphytes might contribute to a buildup of primary inoculum, establishment of new infection centers, and enhance the survival of these bean pathogens (5, 9). Epiphytes of bean blight bacteria also could contribute to the contamination of foliar-feeding insects collected from apparently healthy bean plantings. Whether these epiphytic bacteria can infect bean leaf cells damaged by insects during feeding remains unknown and is an area that merits additional investigation.

The effect of insect transmission on long-distance spread of xanthomonad pathogens of bean and cowpea is unknown. Strong winds or wind-driven rains may transport bacterial blight-infested insects within and among plantings of susceptible crops, and facilitate the spread of bacteria and the establishment of new infection foci. Plant pathogenic xanthomonads survived on live, artificially infested insects for up to 19 days. Bacteria also may survive extended periods on naturally infested insects or contaminated feces, possibly in a hypobiotic state (6). Whether these hypobiotic cells could serve as a source of primary inoculum is unknown.

Of the two natural vectors described in this study, *D. abbreviata* is a large, slow-moving beetle that seldom flies, whereas *C. ruficornis* is a smaller, but much more

active insect that flies frequently, especially when disturbed. In bean and cowpea plantings, populations of *C. ruficornis* also tend to increase much more rapidly than those of *D. abbreviata* (Vakili, unpublished). These characteristics of *C. ruficornis*, in addition the results of our field and inoculation studies, suggest that it is the predominant vector of *Xanthomonas* pathogens of bean and cowpea under field conditions in Puerto Rico. However, in other countries of tropical America the role of these and other foliar pests (1) in the dissemination, transmission, and survival of bean blight bacteria may be different.

## LITERATURE CITED

- BROWN, A. L. 1972. Plant pests of importance to the Caribbean. F.A.O. Office for the Caribbean Region, Port-of-Spain, Trinidad & Tobago, C.A. 91 p.
- BUCHANAN, R. E., and N. E. GIBBONS, eds. 1974. Bergey's manual of determinative bacteriology, 8th ed. Williams and Wilkins, Baltimore, 1,246 p.
- BUDDENHAGEN, I. W., and T. A. ELSASSER. 1962. An insect-spread bacterial wilt epiphytic of Bluggoe banana. *Nature* 194:164-165.
- HAYWARD, A. C., and J. M. WATERSTON. 1965. *Xanthomonas phaseoli*, No. 48 in Descriptions of pathogenic fungi and bacteria. Commonw. Mycol. Inst., Kew, Surrey, England, 2 p.
- IEBEN, C. 1963. Epiphytic microorganisms in relation to plant disease. *Annu. Rev. Phytopathol.* 3:209-230.
- IEBEN, C. 1974. Survival of plant pathogenic bacteria. *Ohio Agric. Res. Devel. Center Spec. Circ.* 100, 21 p.
- SACKETT, W. G. 1905. Some bacterial diseases of plants prevalent in Michigan. Pages 205-220. *Mich. Agric. Exp. Stn. Bull.* 230.
- SAETTLER, A. W. 1971. Seedling injection as an aid in identifying bean blight bacteria. *Plant Dis. Rep.* 55:703-706.
- SCHUSTER, M. L., and D. P. COYNE. 1975. Survival factors of plant pathogenic bacteria. *Nebr. Agric. Exp. Stn. Res. Bull.* 268, 53 p.
- VAKILI, N. G., W. J. KAISER, J. E. PEREZ, and A. CORTES-MONLLOR. 1975. Bacterial blight of beans caused by two *Xanthomonas* pathogenic types from Puerto Rico. *Phytopathology* 65:401-403.
- ZAUMEYER, W. J., and H. R. THOMAS. 1957. A monographic study of bean diseases and methods for their control. U.S. Dep. Agric. Tech. Bull. 868, 255 p.

p	
0.59	
0.99	0.8
0.65	0.3
0.98	0.2

treatments a-  
damaged.  
for 24 hr.  
plants for 24 hr