Synergy between Achromobacter sp. and Pseudomonas phaseolicola Resulting in Increased Disease

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ABSTRACT

A bacterium frequently associated with *Pseudomonas phaseolicola* in culture was a mild pathogen of bean. Physiological-morphological characteristics and a GC ratio of 41.8% indicated that this was an *Achromobacter* sp. The number of lesions incited by *P. phaseolicola* increased from 2- to nearly 4-fold when inocula were mixed with *Achromobacter* sp. Disease enhancement was observed regardless of whether inoculation was by infiltration, mechanical abrasion, or light spray, and whether *Achromobacter* was inoculated simultaneously or prior to inoculation with *P. phaseolicola*. All *Achromobacter* strains tested and one strain of *P. syringae* enhanced infections of *P. phaseolicola*. In contrast, *P. marginalis* and *P. fluorescens* decreased the number of lesions when mixed with *P. phaseolicola*. *P. phaseolicola* multiplied logarithmically in bean leaves following a 12-15 hr lag phase. The amount of growth, however, was greater when the *Achromobacter* sp. was added to the inoculum. Although populations of the *Achromobacter* remained at a relatively low steady rate when singularly inoculated into primary leaves, the bacterium became systemic and was later isolated from noninoculated, symptomless plant parts.

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Additional key words: virulence, pathogenicity, bean.

An unidentified bacterium was frequently detected in cultures of *Pseudomonas phaseolicola* sent to our laboratory during a survey of their nutritional and pathogenic capabilities (24). *P. phaseolicola* was difficult to purify because the two bacteria were closely associated in apparently single colonies, and the second bacterium often could not be detected until after the cultures had been incubated for several wk or longer.

When bean leaves were inoculated with a mixed culture of *P. phaseolicola* and the unidentified bacterium, or when the unidentified bacterium was inoculated alone, an unusual mottled chlorosis occurred on the primary leaves. The unidentified bacterium was consistently reisolated from the chlorotic areas. Because of its association with *P. phaseolicola* and the difficulty in eliminating it from the cultures, the unidentified bacterium was termed an associate.

The preliminary findings suggested the possibility that the associate might be part of the common microflora of bean leaves which could possibly influence the halo blight disease. This seemed an interesting area to explore since plants harbor an internal microflora (3, 29), and a diverse epiphytic population of bacteria (18). Although their role in plant disease is obscure, it is inconceivable that many of these bacteria do not substantially influence disease by their metabolic activities. They are almost universally present in lesions incited by fungi and other pathogenic agents. Walker (34) aptly stated that "it is not correct to refer to a given microorganism as the cause of a given disease, since this implies that it is the sole cause," and suggested the use of the terms incitant or causal organism as they imply that the organism is part of the causal complex.

This paper reports on the identification of a bacterium associated with *P. phaseolicola* and its capacity to increase the severity of the halo blight disease of bean. For comparative purposes, the effect of several pathogenic and saprophytic pseudomonads on disease when mixed with *P. phaseolicola* were also investigated.

MATERIALS AND METHODS.—*Identification of the associate*.— Morphological and physiological characteristics were determined by standard methods (25), unless otherwise stated. Tests for oxidase and for the presence of poly-DL-β -hydroxybutyrate granules were conducted according to Stanier et al. (28). The method of Hugh and Leifson (12) was used to differentiate between oxidative and fermentative utilization of sugars. Nutritional tests were performed as described by Sands et al. (23). Hydrolysis of polypectate was tested by the method of Starr (30) as modified by Sands (23). Arginine dihydrolase production (32) was measured by using medium 2A. The hypersensitive reaction of tobacco leaves (*Nicotiana tabacum* L.) was tested by infiltrating leaves with the bacterium (16). The nucleotide base composition of DNA (moles percent cytosine+guanine) was determined by M. Mandel (Univ. Texas, Houston) using a CsCl density gradient.

Growth of plants.—Phaseolus vulgaris L. 'Red Kidney', 'Pinto', and 'Tendercrop' beans were grown in 7.5-cm diam pots containing U.C. mix (2) on greenhouse benches and illuminated with two 'Gro-Lux' fluorescent tubes for 14 hr/day in addition to available daylight. Plants were grown at 26±4 C, watered by sub-irrigation, and selected for uniformity 24 hr after the crookneck stage (7-10 days after planting). In all experiments, primary leaves were inoculated before trifoliate leaves had begun to develop. Inocula were prepared from 18- to 24-hr-old cultures by measuring turbidity with a Klett-Summerson colorimeter and relating readings to the viable numbers of cells (colony count) using previously determined curves.

Inoculation procedures.— Inoculation was usually by vacuum infiltration (24) since the results were highly reproducible. No macroscopic damage from the treatment was detected and leaves of control plants infiltrated with sterile, distilled water appeared normal throughout the experiment. Lesion numbers, counted 7 days after inoculation, were related to leaf area by measuring traced areas of detached leaves with a planimeter. In mixed inoculation, the inoculum concentration of *P. phaseolicola* (10^5 cells/ml) was maintained constant while the concentration of the associate was varied from 10^1 to 10^6 cells/ml. For a control, *P. phaseolicola* was inoculated alone.

Tests were also made to determine the effect of inoculating the associate bacterium 5 hr before inoculation of *P.*

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phaseolicola. The associate was infiltrated into the primary leaves of one group of plants and distilled water was infiltrated into a second group of plants. Five hr later, both groups were infiltrated with an equal concentration of the pathogen. In other experiments, different bacteria, including *P. marginalis*, *P. fluorescens*, and *P. syringae* were individually substituted for the associate in mixed inoculations.

Inoculation by mechanical abrasion was used as a comparative inoculation technique. Upper surfaces of primary leaves of Red Kidney were dusted lightly with corundum. A cotton swab was dipped into an inoculum of 10^6 cells/ml of *P. phaseolicola* and 10^8 cells/ml of the associate and rubbed gently over leaf surfaces. Lesions on the primary leaves of four plants were counted as previously described.

To more closely simulate natural conditions, a mixed inoculum of 10^8 cells/ml of *P. phaseolicola* and 10^8 cells/ml of the associate were gently sprayed to run-off onto leaf surfaces. This treatment did not cause internal soaking of the tissues, and touching or wounding of leaf surfaces was avoided so that bacteria would be required to enter through stomata. Plants were placed in humidity chambers for 24 hr, then returned to the greenhouse bench. Lesions were counted on primary leaves of four plants, as described.

Isolations of the associate from bean leaves.— To determine whether the associate was part of the natural epiphytic population on bean leaves in the greenhouse, leaves were sampled throughout the year. Bean leaf prints were made on tetrazolium chloride medium (14), which was used to identify colonies with varying abilities to oxidize the substrate. Those with a similar morphology and color typical of known associate isolates were selected from mixed populations on these plates and restreaked on YDCP medium (19) and a medium containing 0.3% yeast extract, 2% glucose, 0.5% peptone, and 2% ager (Difco) (YDP). Forty-five separate isolates with colony morphologies similar to those of the known associate bacterium were tested for the capacity to produce a mottled chlorosis similar to that of the known associate controls. Cell suspensions of the different isolates were inoculated into the primary leaves of Red Kidney plants using the mechanical abrasion method.

Multiplication of bacteria within bean leaf tissues.— The multiplication in leaf tissues of *P. phaseolicola* strain HB 20, associate strains AB 1a, AB 4a, and AB 21c and a *P. fluorescens* strain was determined. In each case, inoculum containing 0.5×10^4 cells/ml of the test bacterium was infiltrated into the primary leaves of 30 Red Kidney plants. Mixed inoculations were made by infiltrating a suspension containing both *P. phaseolicola* (0.5×10^4 cells/ml) and the associate (0.5×10^4 cells/ml). At time intervals of 2, 12, 15, 18, 24, 28, 72, 96, 120, and 144 hr, leaves were detached from three plants, and a 10-mm diam disk was cut with a sterile cork borer from the centers of the primary leaves to one side of the midrib. Disks were triturated immediately in a mortar with 10 ml sterile saline (0.85%). A 10-fold dilution series was made in saline, with mechanical mixing for 1 min between each transfer. A 0.1-ml sample of each of the last three dilutions in the series was spread onto the surface of YDP medium, using a bent

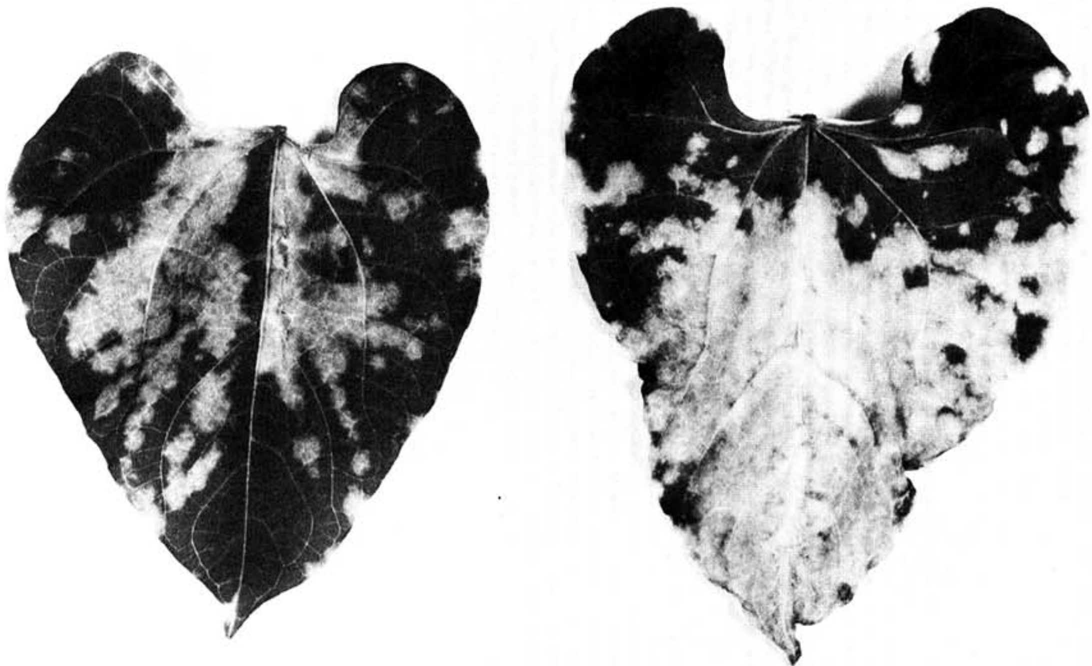

Fig. 1. Primary leaves of Red Kidney bean showing mottled chlorosis caused by *Achromobacter* strain AB 21c. Leaves were inoculated using the mechanical abrasion method with corundum. All *Achromobacter* strains tested produced a similar chlorosis.

glass rod and a mechanical turn-table. Three replications were made for each dilution. Plates were incubated at 27 C, and the dilution which yielded 80 to 150 cells/plate was counted after 24 hr.

Data for all experiments were statistically evaluated by analysis of variance and calculation of standard error.

RESULTS.—*Identification of the associate.*—The associate bacterium is an aerobic, gram-negative, nonspore-forming rod with peritrichous flagellation. Colonies are cream-colored, circular, with rhizoid protrusions at the margins on YDP and YDCP media. Some strains lacked protrusions and grew with undulated margins. It is negative for oxidase, starch hydrolysis, and indole production, and litmus milk turns slightly acid under anaerobic conditions. It produces acid on YDCP and is not fluorescent on King's B medium (15). It produces catalase, pectinase, xylanase; liquifies gelatin; utilizes xylan, glucan, araban, arabinogalactan, and mannan as single carbon sources. The bacterium grows on mineral media containing ammonium salts or nitrate, and converts nitrates to nitrites, although this is not readily apparent because nitrites do not accumulate. Peptone is utilized as a combined nitrogen and carbon source, and a pellicle forms on nutrient broth. It does not grow on eosine methylene blue agar or on a medium specific for *Erwinia* sp. (20). There is no serological relationship to *Erwinia* sp. or *P. phaseolicola*. No growth or crystal formation occurs on poly-DL-β-hydroxybutyrate. The bacterium rots potato slices but not carrot, grows well at 41 C but not at 4 C. It does not cause a hypersensitive reaction on tobacco above 25 C, but induces a weak response at 20 C. It has a mean G+C content of 41.8 moles percent. The following characters were provided by D. C. Sands (*unpublished*) who tested the capacity of this bacterium to utilize various carbon sources in a study of the nutrition of *Pseudomonas*. It grows on fructose, galactose, ribose, mannose, xylose, L(+)arabinose, trehalose, melibiose, raffinose, cellobiose, and sucrose, but not on rhamnose, fucose, D(−)arabinose, or lactose. It grows on succinate, fumarate, α-ketoglutarate, D-gluconate, citrate, and pyruvate, but not on lactic, acetic, malonic, 2-ketogluconic, or maleic acids. It grows on α- and β-methyl-D-glucosides, salicin, and amygdalin, but not on arbutin, esculin, phloridzin, inulin, or chitin. It grows on mannitol and glycerol, but not on methanol, ethanol, propanol, inositol, sorbitol, erythritol, or phenol. It is negative for arginine dihydrolase and urease.

On the basis of these characters, the bacterium was placed in the genus *Achromobacter*. This designation was difficult to make because of the rather confused state of the literature available on the genus. A specific epithet could not be given because the bacterium differed in several important characteristics from the species described in Bergey's Manual of Determinative Bacteriology (5). Although its characters correspond closely with the type species, *A. liquefaciens*, it differed from the descriptions of Tulecke et al. (33) and Eisenberg (7) by growing well at 37 C, causing litmus milk to turn slightly acid, and reducing nitrates to nitrites. Identification was further complicated because the description of Tulecke disagreed with an earlier description by Rush (22) and the original type species had evidently been lost. The G+C base composition of the present *Achromobacter* (41.8%) closely corresponds to the determination reported for *A. liquefaciens* (41%) by Citarella and Colwell (6). *A. liquefaciens* was described as an airborne contaminant of plant tissues (33). The present *Achromobacter* may likewise be present in natural populations of bean leaf tissue but the frequency of its occurrence is unknown because field studies were not undertaken.

Inoculations with Achromobacter.—*Achromobacter* sp. caused a mottled chlorosis on bean leaves when inoculated with abrasion (Fig. 1). This reaction was clearly distinguished from halo blight because the chlorosis was diffuse and was not accompanied by watersoaked lesions. Mottling did not occur when leaves were vacuum-infiltrated or lightly sprayed without wounding.

The number of lesions/cm^2 on Red Kidney leaves caused by *P. phaseolicola* strain HB 20 increased (Fig. 2) when *Achromobacter* AB 1a was infiltrated simultaneously with *P. phaseolicola*. As the concentration of *Achromobacter* increased from 10^4 to 10^6 cells/ml, there was a 2- to nearly 4-fold enhancement of disease severity. Highly significant differences ($P=0.01$) occurred between mixed and single inoculations when the *Achromobacter* concentration was above 10^4 cells/ml. Below this concentration, disease enhancement rarely occurred, indicating that a critical concentration was necessary before the enhancement effect could be measured. Nearly identical results were obtained in subsequent experiments using *P. phaseolicola* (HB20) with a different *Achromobacter* strain (AB 4a), and again using a different strain of the *P. phaseolicola* (HB 28) with *Achromobacter* strain AB 1a. Enhancement of disease also was obtained in two additional experiments when mixed inocula containing 10^6 cells/ml of *Achromobacter* (AB 4b) and 10^5 cells/ml of *P. phaseolicola* (HB 20) were used. Enhancement of disease severity was 1.4-fold in experi-

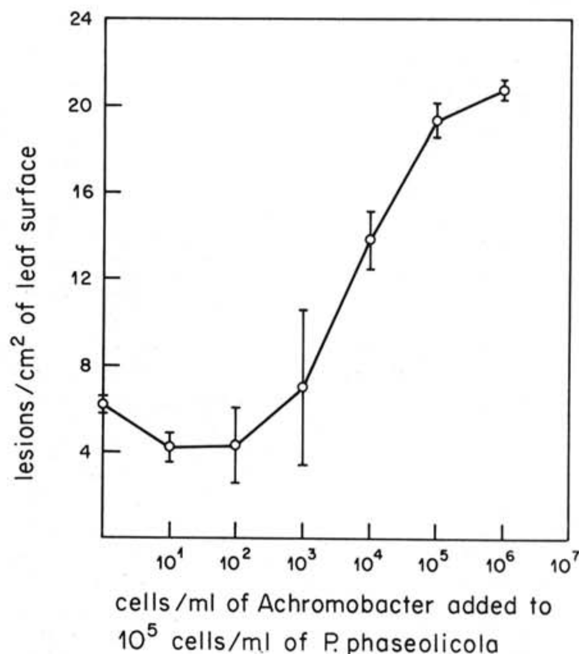

Fig. 2. Effect of *Achromobacter* strain AB 1a on lesion numbers caused by *Pseudomonas phaseolicola* strain HB 20. Each value is the mean of four replications. Bracketed vertical lines indicate standard error.

TABLE 1. Effect of *Achromobacter* strains on lesion numbers caused by *Pseudomonas phaseolicola* on 'Red Kidney' beans

Trial	Inoculum[a]	Lesions/cm^2
1	*P. phaseolicola* HB 20	17.2±3.1[c]
	P. phaseolicola HB 20+*Achromobacter* AB 4a	52.4±9.2**
2	*P. phaseolicola* HB 20	26.6±4.0
	P. phaseolicola HB 20+*Achromobacter* AB 4a[b]	88.7±8.9**
3	*P. phaseolicola* HB 20	19.2±2.9
	P. phaseolicola HB 20+*Achromobacter* AB 21c	27.4±1.4**
4	*P. phaseolicola* HB 20	15.4±2.6
	P. phaseolicola HB 20+*Achromobacter* AB 21c[b]	24.0±2.7*

[a]The concentration of inoculum for *P. phaseolicola* and *Achromobacter* was 10^5 and 10^6 cells/ml, respectively.

[b]Bean plants were inoculated with *Achromobacter* 5 hr before *P. phaseolicola* was introduced.

[c]**=difference significant at 1% level;*=difference significant at 5% level.

TABLE 2. Effect of *Pseudomonas fluorescens* and *P. marginalis* on lesion numbers caused by *P. phaseolicola* strains HB 20 and HB 28 on 'Red Kidney' beans

Inoculum[a]	Lesions/cm^2
P. phaseolicola HB 20	2.9±0.6[b]
P. phaseolicola HB 20+*P. fluorescens*	0.9±0.2*[c]
P. phaseolicola HB 20+*P. marginalis*	1.9±0.2 NS
P. phaseolicola HB 28	5.9±1.5
P. phaseolicola HB 28+*P. fluorescens*	1.3±0.3*
P. phaseolicola HB 28+*P. marginalis*	1.6±0.3*

[a]The concentration of inoculum for *P. phaseolicola* and other bacteria was 10^5 and 10^6 cells/ml, respectively.

[b]Mean of eight leaves.

[c]*=difference significant at 5% level; NS=not significant.

ment 1 (P=0.05) and 2.8-fold in experiment 2 (P=0.01).

Simultaneous infiltration of *Achromobacter* and *P. phaseolicola* was also made with bean varieties Tendercrop and Pinto which are considerably more susceptible and less susceptible (respectively) than Red Kidney to halo blight infections. The results were similar to those found with Red Kidney except that disease enhancement with Tendercrop required 10^5 cells/ml of *Achromobacter* for a 2-fold increase (P=0.01) whereas only 10^4 cells/ml of this bacterium were required to obtain the effect on Red Kidney and Pinto.

When *Achromobacter* isolates were infiltrated into the leaves 5 hr prior to infiltration of *P. phaseolicola*, the increase in disease severity was essentially the same as when the two bacteria were infiltrated simultaneously (Table 1). A 3.0- to 3.4-fold increase was observed when *Achromobacter* strain AB 4a was used as the associate, and a 1.4- to 1.5-fold increase occurred with strain AB 21c.

When Red Kidney plants were inoculated by the mechanical abrasion technique with mixed inocula of *Achromobacter* (AB la) and *P. phaseolicola* (HB 20), the number of lesions/cm^2 was again ca. 2-fold higher than it was when *P. phaseolicola* was inoculated alone. The results were similar in two separate experiments and differences were significant at the 1% level. In a light-spray inoculation the combination of *Achromobacter* with *P. phaseolicola* also incited a 2-fold increase in lesion number on both Red Kidney and Pinto leaves. Results were similar with *Achromobacter* strains AB 4b and AB 21c.

Effect of other bacteria on disease severity in mixed inoculations.— For comparative purposes, the pathogens *P. syringae* and *P. marginalis* and the saprophyte *P. fluorescens* were added to inoculum of *P. phaseolicola*. Both *P. marginalis* and *P. fluorescens* significantly decreased the number of lesions produced by *P. phaseolicola* strains HB 20 and HB 28 (Table 2), whereas *P. syringae* (540a) caused a significant increase (Table 3). There was nearly a linear relationship between the number of lesions produced and the amount of *P. syringae* inoculum added to *P. phaseolicola*. No watersoaking occurred when plants were infiltrated with *P. syringae* alone. *P. syringae* 540a was originally isolated from bean, but had lost virulence in culture and only produced small dry necrotic lesions on bean leaves.

Isolations of Achromobacter from bean leaves.— Attempts to isolate *Achromobacter* from bean leaf surfaces in the greenhouse were inconclusive. Hundreds of colonies were examined, and many exhibited a morphology and pigmentation similar to identified *Achromobacter* strains. However, none of the 45 isolates purified and reinoculated onto Red Kidney bean leaves incited the typical mottled chlorosis produced by known *Achromobacter* strains. Four bacterial isolates incited a slight distortion or marginal chlorosis, but the majority of the isolates produced no visible symptoms.

Multiplication of bacteria within bean leaf tissues.— *P. phaseolicola* multiplied logarithmically after a lag phase of 12-15 hr when infiltrated alone into leaf tissues of Red Kidney bean (Fig. 3). Two repetitions of the same experiment gave nearly identical results. *P. fluorescens* declined immediately after inoculation and was not detected in bean leaf tissues after 96 hr. Populations of *Achromobacter* strain AB 4a remained at a steady state during the 144-hr test period (Fig. 3), showing neither a rapid decline nor a high rate of multiplication. *Achromobacter* strains AB la and AB 21c produced similar growth patterns. These strains were later isolated from 14- and 21-day-old primary leaves exhibiting symptoms of mottled chlorosis. The bacteria were also isolated from green areas of primary leaves, from noninoculated trifoliate leaves showing no symptoms of mottled chlorosis, and from petioles of both primary and trifoliate leaves. The leaves and petioles were treated with a 0.5% solution of sodium hypochlorite for 1 min before isolations were made. *Achromobacter* occasionally caused small necrotic flecks on the trifoliate leaves which were invaded following inoculation of the primary leaves.

The populations of *Achromobacter* did not change appreciably when *P. phaseolicola* was added to the inoculum (Fig. 3). In contrast, however, *Achromobacter* affected the growth curve of *P. phaseolicola*, shortening the lag period so that it was not detected. The resultant increase in population of *P. phaseolicola* at the time of lesion formation was accompanied by a corresponding increase in the numbers of lesions/cm^2. Thus, the enhance-

ment effect appeared to be related to higher populations of the pathogen within leaf tissues at the time of lesion formation.

DISCUSSION.— The capacity of certain isolates of *Achromobacter* to increase the severity of halo blight disease was unexpected since most studies using combinations of bacteria have resulted in less disease (9, 11, 18). For example, Teliz-Ortiz and Burkholder (31) found that *P. fluorescens* isolated from halo blight diseased tissues was antagonistic to *P. phaseolicola* in culture and caused decreased disease severity when present in mixed inoculum, a finding similar to ours. Omer and Wood (21), using combinations of *P. phaseolicola* race 1 and race 2, further found that the presence of an avirulent race in mixed inoculum decreased the numbers of lesions caused by a virulent race.

The ease by which large numbers of bacteria and combinations thereof can be intromitted into plant parts increases the opportunity for obtaining interesting laboratory anomalies with little relevance to nature. With this in mind, we used a variety of inoculation techniques, plant varieties, and bacterial strains with varying amounts of inoculum to determine whether or not the effect of *Achromobacter* in contributing to increased lesion numbers by *P. phaseolicola* was real and a probable occurrence in the field. In all tests, disease enhancement occurred. The most satisfying result, however, was the obtaining of disease enhancement by lightly spraying bean leaves with a mixed inoculum, thereby enabling the bacteria to enter the tissues through natural openings.

That populations of *Achromobacter* do not decline in the plant is further evidence that *Achromobacter* is not alien to the plant environment. In addition, *Achromobacter* demonstrated invasive properties in that it was isolated from noninoculated, symptomless trifoliate leaves 21 days after inoculation of primary leaves. On occasion it also incited small necrotic flecks on trifoliate leaves which had been invaded. The *Achromobacter* isolates, therefore, are considered mild pathogens, which normally cause little visible damage. The *P. syringae* which also contributed to an increase in disease severity is probably mildly pathogenic and causes some injury.

Information regarding the association of other bacteria with *P. phaseolicola* in halo blight disease is scarce. In 1944, Jensen and Livingston (13) mentioned that new strains of *P. phaseolicola* occurred continuously in culture and produced a marked mottling on leaves near the growing point. Whether these workers were referring to bacteria similar to those investigated here cannot be determined, as no further discussion of the subject was found. However, it is possible that the so-called new strains were merely the result of a mixed culture where two bacteria were difficult to separate. In other work, Adam and Pugsley (1) reported that a yellow bacterium associated with *P. phaseolicola* in diseased bean stems, leaves, and pods decreased the severity of halo blight disease. Although the physiological description of the yellow bacterium was brief, it is apparent that it was not an *Achromobacter* sp.

Many investigators have pointed out that virulence of a pathogen is directly related to its ability to multiply within host tissues (10, 17, 26). Ercolani and Crosse (8) proposed that growth of bacteria to certain population levels within host tissues was a prerequisite for development of disease symptoms and reported that a threshold level slightly less than 10^7 cells/cm^2 of leaf tissue was necessary for the production of watersoaked symptoms by *P. phaseolicola*. In addition, the number of watersoaked symptoms depended on the amount of viable cells in the initial inoculum and the capacity of these cells to increase beyond the threshold value for symptom production. Thus, the increased number of watersoaked lesions observed when *Achromobacter* is added to *P. phaseolicola* inocula may be explained by the

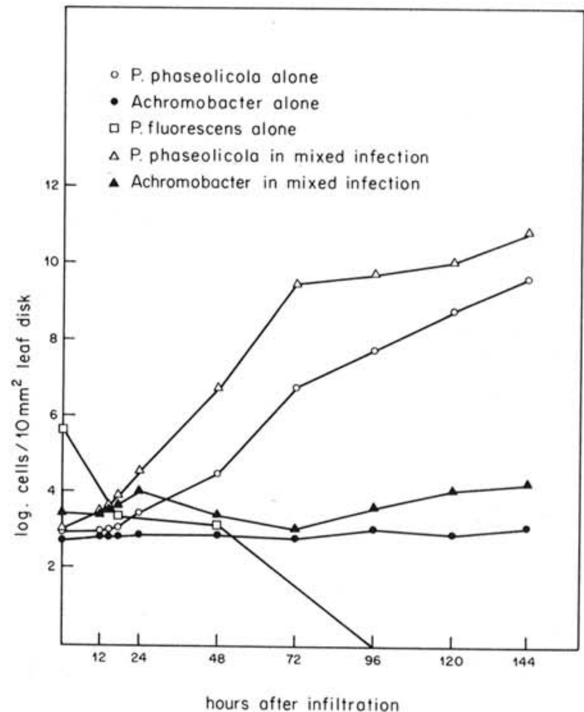

Fig. 3. Multiplication of bacteria within primary leaves of 'Red Kidney' beans when inoculated singly and in combinations. Leaves were infiltrated with *Pseudomonas phaseolicola* strain HB 20, *Achromobacter* strain AB 4a, or *P. fluorescens*. In mixed inoculations, *P. phaseolicola* and *Achromobacter* (strain AB 4a) were infiltrated simultaneously. Each value is the mean of three replicates.

TABLE 3. Effect of *Pseudomonas syringae* (strain 540a) on numbers of lesions caused by *P. phaseolicola* (strain HB 20) on 'Red Kidney' bean.

P. syringae (cells/ml) added to *P. phaseolicola* inoculum[a]	Lesions/cm^2
Control[a]	4.9±0.8[b]
10	6.2±0.8 NS
10^2	6.9±0.7 NS
10^3	7.4±0.3**
10^4	7.5±0.9**
10^5	6.9±0.9**
10^6	9.2±1.3**

[a] Inoculum of *P. phaseolicola* was 10^5 cells/ml.
[b] **=difference significant at 1% level; NS=not significant.

enhanced capacity of *P. phaseolicola* to multiply within host tissues.

The mechanism by which *Achromobacter* causes increased multiplication of *P. phaseolicola* in the intercellular spaces is a subject for further investigation. It may alter the leaf microenvironment by elaborating specific degradative enzymes which act on host compounds, thereby releasing metabolizable substrates for *P. phaseolicola*, or it might cause structural changes in the cell wall, thereby promoting the efficiency of *P. phaseolicola* in reaching susceptible sites.

Interactions between plant pathogens and resident plant microflora have not received much attention by plant pathologists. In contrast, the importance of saprophytic microflora has been recognized in medical science as vital in preventing disease and also as dangerous secondaries following a primary infection. A clear understanding of the role of associate microorganisms is needed to determine the full potential of a plant pathogen to cause disease. Brathwaite and Dickey (4), for example, have demonstrated that a *Corynebacterium* sp., a bacterium commonly found in carnations, significantly increased the rate of wilting and the extent of basal stem rot symptoms of carnation when present in mixed infections with *P. caryophylli*, while alone it caused no visible symptoms. Stanghellini (27) reported that potatoes harbor soft rot bacteria internally and that the soft rot disease can be triggered by several fungi penetrating the potato tissues.

These studies thus emphasize the importance of considering the activities of other microorganisms commonly found in or on the host when investigating infection processes. It is likely that a number of saprophytes and unrecognized mild pathogens occur in plants and ordinarily are relatively inoculous. However, when in association with a virulent pathogen, the interaction may result in increased disease severity.

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