Microorganisms Associated with Bottom Rot of Lettuce Grown on Organic Soil in New York State

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ABSTRACT

Rhizoctonia solani, Fusarium oxysporum, Mucor sp., *Trichoderma* sp. and *Alternaria* sp. frequently were isolated from leaves and heads of lettuce grown on organic soils in New York and exhibiting initial symptoms of bottom rot. *R. solani* consistently was isolated from infected tissue at all stages of symptom development. It was the only fungus of the group pathogenic to lettuce when nonwounded detached leaves were inoculated. Under field conditions *Erwinia carotovora, Pseudomonas marginalis,* and *P. fluorescens* frequently were present in addition to *R. solani* on plants with extensive maceration and vein-browning of heads. In pathogenicity tests using detached lettuce leaves, all bacterial isolates required a wound before infection occurred. When excised leaves with lesions formed by *R. solani* were inoculated with soft rot bacteria, the resulting rate of decay was greater than on leaves infected with only *R. solani*. This suggests that *R. solani* frequently plays a role in providing a mode of entry for the soft rot bacteria under field conditions.

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Additional key words: Rhizoctonia solani, Lactuca sativa, soft rot bacteria.

Lettuce (*Lactuca sativa* L.) is one of the major vegetable crops grown on organic soils in New York State. Growers seldom practice crop rotation and frequently grow two crops of lettuce in the same field each year. Monoculture of lettuce has increased the prevalence and severity of diseases caused by soil-borne pathogens, and at present bottom rot is the most important disease. In New York, Townsend (24) estimated that losses each year due to bottom rot reached 30% of the crop, and that during wet periods losses of 50 to 75% of the maturing crop were encountered. In recent years we have observed similar losses because of this disease. Bottom rot was first described by Stone and Smith (21) and the causal agent was identified as *Rhizoctonia solani* Kühn (8). On organic soils in New York, bottom rot is characterized by a basal decay of lettuce plants that are headed and nearly mature. Symptoms first appear as discrete lesions on the leaf parts in contact with the soil. During warm, humid periods the lesions expand into a decay that rapidly spreads to the adjoining leaves until much of the head becomes a decayed mass. The rapid slimy decay characteristic of the advanced stages of bottom rot suggests that soft rot bacteria or decay fungi may be involved in a complex with *R. solani,* causing a rapid maceration of lettuce tissue under field conditions. Soft rot bacteria and decay fungi commonly invade lettuce tissue after infection by primary pathogens (19, 27). The interactions of soft rot bacteria and decay fungi on lettuce has received little attention. We suspect that soft rot bacteria and fungi readily invade the initial lesions produced by *R. solani* and contribute to the type and rate of symptom development. Because bottom rot continues to be a major problem for lettuce growers in New York, this study was undertaken to gain new information on the biology of the disease.

MATERIALS AND METHODS.—Several lettuce fields in Oswego County, New York planted to the cultivar 'Minetto' were examined at weekly intervals during 1972 to study the time sequence of botton rot development. Observations were initiated in May and terminated in September. Infected leaf and head samples also were collected at weekly intervals to investigate the organisms associated with the different stages of disease development. The diseased material was collected in plastic bags, transported to the laboratory, refrigerated at 1 C, and processed within 24 hours. Samples were washed-free of organic soil and divided into three categories based on the degree of symptom development: initial discrete lesions, expanding lesions with diffuse margins, and advanced stages of decay.

Isolations and identification.—For isolation of fungi, tissue samples were rinsed in one liter of sterile water containing five drops of Tween 20, treated in 0.5% NaOCl (10% aqueous solution of a commercial bleach mix) for 1 minute and rinsed in three changes of sterile water. Sections of tissue 3 mm in diameter were cut from the margins of necrotic areas. These were plated on acidified potato-dextrose agar (five drops 50% lactic acid/100 ml) and modified V-8 juice agar (17) and incubated at 27 C for 3 to 5 days. The total number of colonies of specific fungi that developed were counted and identified following Barnett and Hunter (2), Toussoun and Nelson (23), and Alexopoulos (1). Representative fungal isolates were maintained on potato-dextrose agar (PDA) slants for later pathogenicity tests.

To isolate soft rot bacteria associated with bottom rot, the technique of host plant inoculation (25) selective for pathogenic bacteria was used. Healthy lower leaves and larger leaves of the head were excised from 7- to 8-week-old lettuce plants (cultivar Minetto). Large leaves were cut in half. All leaves were handled with care to minimize injury. The leaves were rinsed in one liter of sterile water containing five drops of Tween 20, surface disinfested in 0.5% NaOCl (10% aqueous solution of commercial bleach) for 5 minutes and rinsed in three changes of sterile water. The disinfested leaves were placed on wire

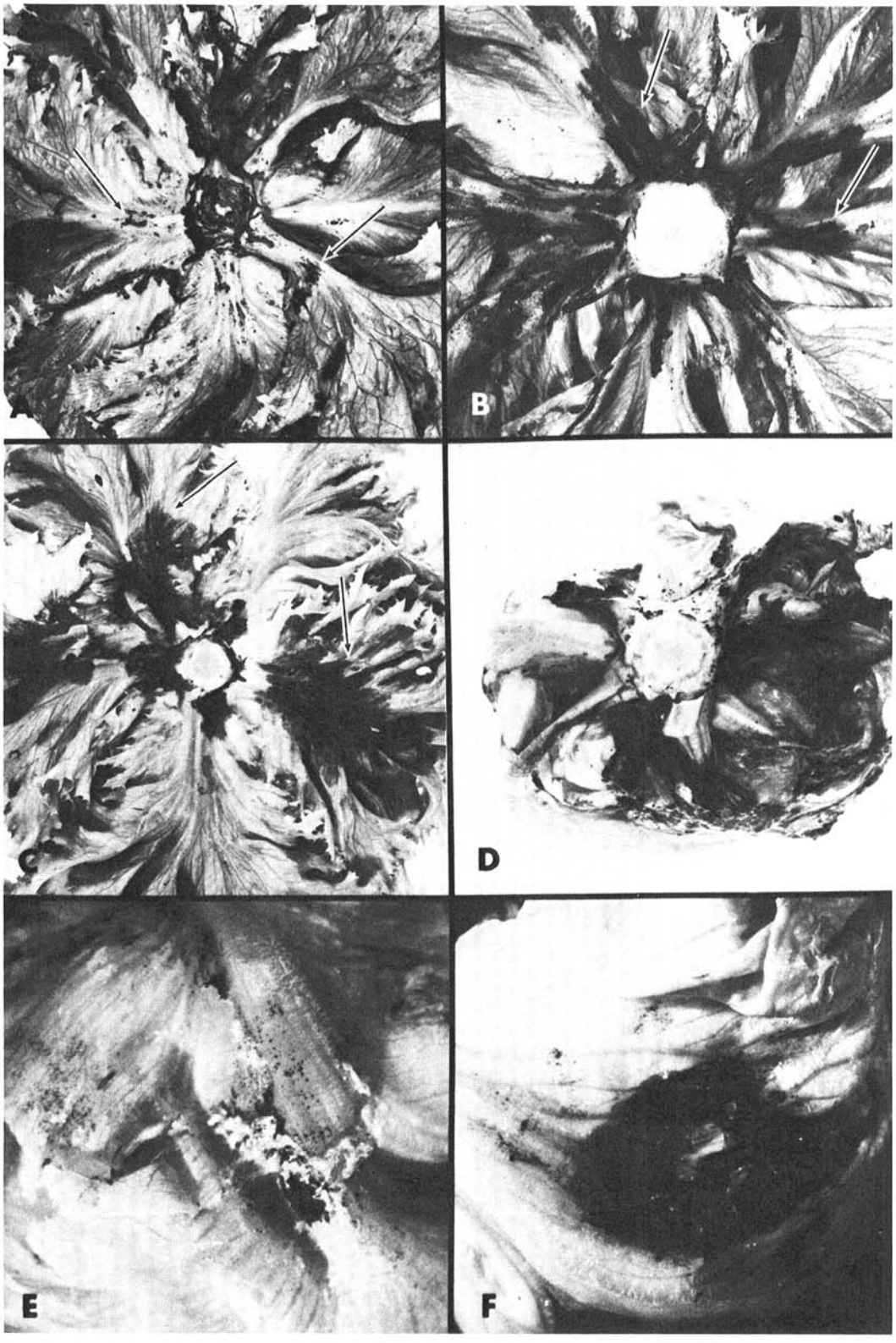

Fig. 1-(A-F). Typical bottom rot symptoms. **A)** Initial lesions on midribs and blades of lettuce leaves; **B)** Expanding dark-colored lesions with diffuse margins; **C)** Decay spreading to leaf lamina; **D)** Advanced stages of head decay; **E)** Interior portion of a decaying head exhibiting dry lesions covered with *R. solani* mycelium; **F)** Interior portion of a decaying head exhibiting a dark, watery soft rot and vein browning.

TABLE 1. Comparison of certain physiological and biochemical characters of *Erwinia carotovora*[a] (*E.c.*) with *Erwinia* spp. isolates from lettuce

Character	*E.c.*	*Erwinia* spp. isolates				
		C-32	S-51	W-62	W-63	W-69
Acid from:						
lactose	+	+	+	+	+	+
glucose or sucrose	−	−	−	−	−	−
Gelatin liquefaction	+	+	+	+	+	+
Indole	+	+	+	+	+	+
Gluconate oxidation	−	−	−	−	−	−
Sucrose-reducing compounds	+	−	−	−	−	−
Methyl red test	+	+	+	+	+	+
Acetylmethyl carbinol	+	+	−	+	+	+
Catalase reaction	+	+	+	+	+	+
Nitrate reduction	+	+	+	+	+	+
Potato rot	+	+	+	+	+	+

[a]Isolate provided by R. S. Dickey, Department of Plant Pathology, Cornell University, Ithaca, New York.

TABLE 2. Comparison of certain physiological and biochemical characters[a] of *Pseudomonas marginalis*[b] with the *Pseudomonas* spp. isolated from lettuce

Isolates	Group	Lev	Ox	Pr	Arg	Tob	2-Kg	Nr	Suc	Gel	Aes	Tyr
P. marginalis		+	+	+	+	−	+	+	+	+	+	[c]
D-23	I	+	+	+	+	−	+	+	+	+	+	−
S-71		+	+	+	+	−	+	+	−	+	−	−
S-74		+	+	+	+	−	+	+	+	+	+	−
S-73	II	+	+	−	+	−	−	+	+	+	+	−
W-53		+	+	−	+	−	−	−	+	+	+	−
C-31	III	−	−	+	−	+	−	−	−	−	−	−
D-31		−	−	+	−	+	−	−	−	−	−	+
S-21		−	−	+	−	+	−	−	−	−	−	−
W-21		−	−	+	−	+	−	−	−	−	−	+

[a]Physiological and biochemical characters: Lev = Levan, Ox = Oxidase, Pr = potato rot, Arg = Arginine, Tob = Tobacco, 2-Kg = 2-Ketogluconate, Nr = Nitrate reduction, Suc = Sucrose, Gel = Gelatin, Aes = Aesculin, Tyr = Tyrosine.
[b]Isolate provided by R. S. Dickey, Department of Plant Pathology, Cornell University, Ithaca, New York.
[c]Not tested.

supports (Fig. 2-C) in sterilized 20.3-cm (8-inch) diameter, covered Pyrex casserole dishes containing about 100 ml of sterile water to maintain a moist environment. Segments of infected tissue from lesion margins or margins of decaying areas were placed over a wounded area on a disinfested leaf with free water added and incubated at 27 C. If a lesion developed after 24 hours, a section of tissue was taken from the lesion margin and placed on a second wounded leaf and incubated for 24 hours. If a lesion developed on the second leaf, a glass rod was rubbed across the lesion margin and the cells adhering to the rod were streaked onto a tetrazolium medium (12). Usually two or three different colony types developed. Sample colonies were picked off and streaked onto the tetrazolium medium a second time to assure purity. Representative isolates were grown on nutrient agar (NA) for 24 hours. Selected isolates were preserved by suspending several loopfuls of cells in 5 ml of sterile distilled water in 20 × 150 mm screw-cap test tubes and maintained at 10 C.

Fourteen bacterial isolates strongly virulent on lettuce, as determined by excised leaf inoculations, were used in identification studies. For comparative purposes, isolates of *Erwinia carotovora* (Jones) Holland and *Pseudomonas marginalis* (Brown) Stevens were obtained from R. S. Dickey, Department of Plant Pathology, Cornell University, Ithaca, New York. Hucker's procedure for gram-staining was utilized (20). Flagellation was determined by the Bailey method (10). The Hugh and Leifson test (13) was used to differentiate oxidative and fermentative metabolism of carbohydrates. The medium of Ayers, Rupp, and Johnson (20) and triple-sugar agar (20) were used to test utilization of carbon compounds. The methyl red test and tests for acetylmethyl carbinol, indole production, catalase activity, and gelatin liquefaction were conducted following procedures described by Dye (9). Pathogenicity on potato slices and tests for gluconate oxidation, production of arginine dehydrogenase, oxidase, tyrosinase and levan, and hydrolysis of aesculin were made following procedures described by Lelliott et al. (15). All media were sterilized by autoclaving at 121 C for 15 min. Inoculum for all tests consisted of cells from 18- to 24-hour cultures on NA slants incubated at 27 C. Incubation temperature for all tests was 27 C.

Pathogenicity tests.—The comparative pathogenicity of representative fungal and bacterial isolates from decaying lettuce was tested using the excised leaf method previously described. For fungi, 3-mm diameter mycelial disks from the margins of cultures growing on PDA or

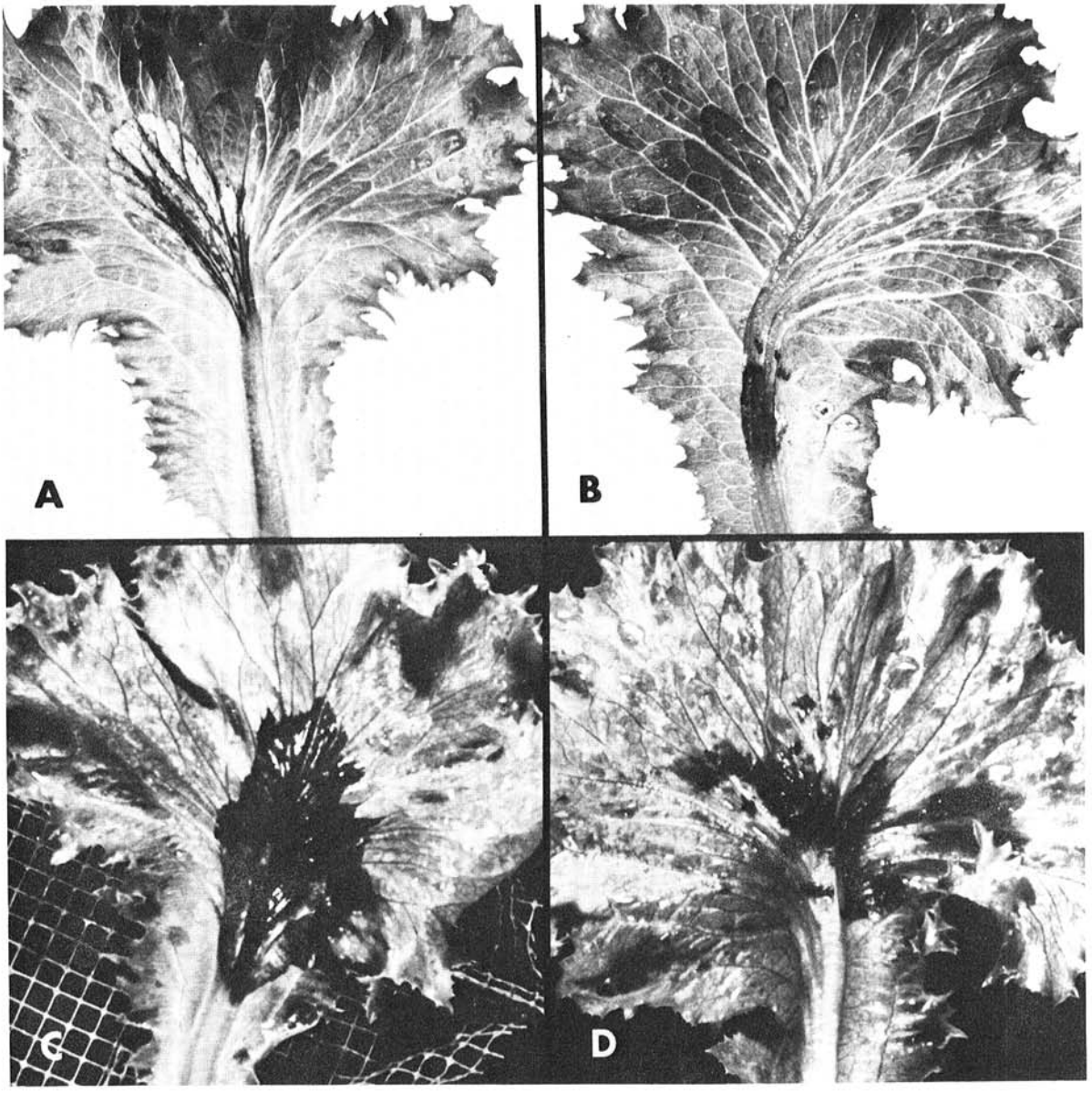

Fig. 2-(A-D). Symptom development on detached lettuce leaves inoculated with *R. solani* and/or soft rot bacteria. **A)** Amount of maceration 48 hours after inoculation with a bacterial isolate; **B)** Symptom development 48 hours after inoculation with *R. solani;* **C)** Symptom development on leaf incubated with *R. solani* for 48 hours and then inoculated with soft rot bacteria and incubated for 48 hours; **D)** Symptom development 4 days after inoculation with *R. solani.*

soil extract agar (11) were placed on either wounded or nonwounded leaves. The bacterial isolates were tested by placing 10^7 cells in 0.2 ml of water on either wounded or nonwounded leaves. Combination inoculations were studied with *R. solani* in combination with each of the other fungi isolated from decaying lettuce. In one test, leaves were inoculated with all five fungi. In *R. solani*-bacteria combinations, leaves were inoculated with *R. solani* and incubated for 48 hours before the bacterial isolate was added.

RESULTS.—*Symptomatology.*—Bottom rot symptoms on field-grown lettuce plants usually appeared 6-7 weeks after seeding or 1-2 weeks before harvest. The first plant parts diseased are the leaf petioles and the midrib portions in contact with the soil (Fig. 1-A). The initial symptoms are rust-colored, well-defined, sunken lesions. Under warm, humid conditions the lesions expand, become darker, and develop diffuse margins (Fig. 1-B). Once commenced, the decay spreads immediately to adjoining leaves in succession until much of the head becomes decayed (Fig. 1-C,D). At this stage the interior portions of the heads may exhibit differences in symptom expression. In some cases (Fig. 1-E) the interior portions exhibit dry lesions covered by mycelium

of *R. solani*. In other cases, a dark watery soft rot is evident (Fig. 1-F) with extensive vein browning. This type of symptom was observed on approximately 40% of the head samples collected.

During wet growing periods, the time interval from the first appearance of lesions to the advanced stages of decay is 5 to 10 days. Under drier conditions, the initial lesions may develop on the lower leaves in contact with the soil, but little or no decay occurs.

Isolations and identification.—*R. solani* and *Fusarium oxysporum* Schlecht. emend. Snyd. & Hans. consistently were isolated from lower lettuce leaves with initial lesions and advancing stages of decay. *Alternaria* sp., *Mucor* sp., and *Trichoderma* sp. also were isolated consistently, but less frequently. Of the fungi, only *R. solani* was isolated from decaying interior portions of the heads in advanced stages of decay.

No pathogenic bacteria were isolated from the initial lesion stages of symptom development. In addition to *R. solani,* soft rot bacteria frequently were present on lettuce heads with extensive maceration and vein browning of heads. Identification studies revealed that two types of gram-negative, rod-shaped bacteria were involved: polar-flagellated, green-fluorescent pseudomonads and peritrichously flagellated, facultative anaerobic Erwinias. The *Erwinia* group was homogeneous whereas the *Pseudomonas* group was divided into three categories, referred to here as groups I, II and III.

Selected physiological and biochemical characteristics of the *Erwinia* group and the known isolate of *E. carotovora* were determined (Table 1). Of 14 bacterial isolates studied from lettuce, five were similar to the known isolate of *E. carotovora* from lettuce.

The characteristics of the *Pseudomonas* groups and the known isolate of *P. marginalis* from lettuce were determined (Table 2). *Pseudomonas* group I consisted of three isolates that were similar to the known isolate of *P. marginalis* from lettuce. One isolate, S-71, differed by not producing acid from sucrose or hydrolyzing aesculin. *Pseudomonas* group II consisted of two isolates that were similar to the description of *P. fluorescens* provided by Lelliott et al. (15). *Pseudomonas* group III consisted of four unidentified green-fluorescent isolates.

Pathogenicity tests.—*R. solani* isolates caused a decay of excised lettuce leaves. Neither *F. oxysporum, Alternaria* sp., *Trichoderma* sp. or *Mucor* sp. alone caused decay or increased the amount of decay when mixed with *R. solani* inoculum. Mixed inoculum, containing all five fungi including *R. solani,* produced decay that was always similar to *R. solani* alone.

All bacterial isolates identified required a wound before decay occurred. When 10^7 cells in 0.2 ml of water were applied to a wound, the tissue was macerated very rapidly (Fig. 2-A). Maceration also occurred on nonwounded detached leaves inoculated with *R. solani,* but at a slower rate (Fig. 2-B). Maceration occurred at a faster rate on nonwounded detached leaves first inoculated with *R. solani* and then with soft rot bacteria added than on leaves infected with *R. solani* alone (Fig. 2-C,D). Such experiments were repeated several times during the course of this study.

DISCUSSION.—Symptom development of bottom rot of lettuce grown on organic soils in New York can be separated into three stages: (i) initial discrete lesions, (ii) advancing lesion margins, and (iii) head decay. During critical study of the organisms associated with the different stages of bottom rot development, it became clear that a disease complex involving *R. solani* and soft rot bacteria was operative. *R. solani* was the only pathogenic fungus isolated from all stages of symptom development. Soft rot bacteria consistently were associated with *R. solani* in the inner portions of decayed heads that were slimy, dark in color, and exhibited extensive vein browning. Of the four species of pathogenic bacteria found associated with bottom rot, *E. carotovora* and *P. marginalis* are the most commonly reported causes of bacterial decay of lettuce under field conditions (3, 5, 6, 7, 16, 22, 26). In addition to decaying leaf tissue, *E. carotovora* has been reported to decay stem tissue (26), but this symptom has not yet been observed in New York. The third species of soft rot bacteria isolated in this study (*Pseudomonas* group II) appears to be *Pseudomonas fluorescens* Migula which rarely has been reported as a plant pathogen (4, 14). The two isolates identified as *P. fluorescens* and the four unidentified soft rot pseudomonads (*Pseudomonas* group III) were strongly virulent when first isolated, but rapidly lost their virulence when maintained in culture. Unidentified soft rot pseudomonads are commonly reported on crop plants and their relationships with known pathogenic species are

Soft rot bacteria appear to function chiefly as important secondaries that assume a major role in modifying symptom development of bottom rot when compared to plants infected with *R. solani* alone. This concept is supported by the results of pathogenicity tests under controlled conditions using excised lettuce leaves. All the bacterial isolates tested required a wound on healthy lettuce leaves before infection occurred. *R. solani,* which penetrates healthy tissue directly, often produced distinct lesions 48 hours after inoculation of healthy leaves. Soft rot bacteria gained ingress through these lesions and caused a slimy, dark colored decay of lettuce tissue similar to that observed in the field. In relating these studies to field conditions, it appears that *R. solani* attacks lettuce leaves when they contact the soil, resulting in a lesion formation on the leaf petioles or midribs. Under moist conditions, the lesions expand into a light brown decay as *R. solani* spreads to the adjoining leaves. Under optimum conditions *R. solani* can decay much of a lettuce head in 7-10 days. However, soft rot bacteria frequently enter the lesions produced by *R. solani* and alter symptom development. Soft rot bacteria are common in organic soil and especially on lower senescing lettuce leaves which are common on 6- to 7-week-old plants (18). Because the lower healthy leaves are in contact with both the soil and the senescing leaves, soft rot bacteria can readily enter the healthy tissue when wounds occur. When *R. solani* infection has occurred, the bacteria under moist conditions can enter the resulting lesion and multiply. When soft rot bacteria become successfully established, a rapid, dark-colored, slimy decay of the head results. In experiments conducted under controlled conditions, the dark coloration was characteristic of tissue infected with both *R. solani* and soft rot bacteria. Under field conditions, plants infected with both organisms can be recognized by the darker color, extensive vein browning and more advanced decay than plants infected with *R. solani* alone. Although *R.*

solani can provide a mode of entry for bacteria in nature, it is possible that the bacteria isolated may function as primary pathogens when they come in contact with a wounded area in otherwise healthy lettuce tissue.

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