Bacterial Blight of Onion, a New Disease Caused by Xanthomonas sp.

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ABSTRACT

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A new disease of green and bulb onion in Hawaii is caused by a previously undescribed *Xanthomonas* sp. Tip dieback, leaf lesions, chlorotic streaks, and, eventually, necrosis of the entire leaf results in stunted plants and undersized bulbs at harvest. The pathogen was isolated from leaf lesions and inoculated onto 15 susceptible onion cultivars. Physiological and biochemical tests showed that the pathogen belonged to the *X. campestris* taxospecies, but that it differed from other *Xanthomonas* nomenspecies of the group in pathogenicity, serology, and in utilization patterns of 122 carbon sources.

A previously unreported leaf spot of onion first was observed on the island of Molokai, Hawaii, in early 1975 on a sweet yellow onion, *Allium cepa* 'Granex 33'. Plants were stunted and tip dieback with premature death of leaves resulted in abnormally small bulbs at harvest (Fig. 1). Leaves had lenticular watersoaked lesions which elongated into chlorotic streaks, usually more prominent on the flat sides of older leaves. On Molokai, undersized bulbs caused severe marketing losses in two successive crops. Subsequently, the disease was observed on the islands of Kauai and Maui. Although onions are grown on Oahu and Hawaii, the disease has not yet been observed in commercial fields there. In a preliminary report (1) the disease was described as a leaf spot caused by a *Xanthomonas* sp. Subsequent isolations revealed that the pathogen also was found in association with extensive blighting of outer leaves. Studies were undertaken to determine whether this *Xanthomonas* sp. was always associated with leaf blight and was primarily responsible for these symptoms.

MATERIALS AND METHODS

Isolation and identification of the pathogen.—Leaf samples were refrigerated in an ice chest immediately after collection from the field. Leaves were immersed in 0.5% sodium hypochlorite for 5 min. Small (1 mm^2) sections were taken from: (i) leaf tips, (ii) borders and centers of lesions, (iii) chlorotic streaks, and (iv) from leaf sheaths and bulbs of diseased and healthy plants. Since *Botrytis* sp., *Alternaria porri,* and *Stemphylium botryosum* are known to cause leaf diseases of onion (15), leaf sections were examined microscopically for the presence of mycelium. Also, tissues were embedded in 2% water agar or potato-dextrose agar (Difco) to recover fungi. The techniques of Ellerbrock and Lorbeer (6) were employed in an attempt to isolate *Botrytis squamosa.*

For bacterial isolations, tissues were ground in 0.2 ml of sterile distilled water (SDW) and loopfuls of the suspension were streaked on a series of media, including Kelman's tetrazolium chloride (TZC) medium (7), yeast-dextrose-calcium carbonate (YDC) (5), King's Medium B (8), Miller and Schroth's *Erwinia* medium (9), and Schaad's medium for *Xanthomonas* (11). Suspected pathogens were restreaked four times successively from single colonies onto fresh TZC plates. Isolates were inoculated into onion leaves, reisolated, and stored in SDW at 8 C. Standard determinative tests (2, 3) were performed on thirteen isolates from representative fields, onion cultivars, and seed sources. Methods of Stanier et al. (13) were used to test growth on carbon sources and to compare results with 22 strains from 10 other *Xanthomonas* nomenspecies.

Pathogenicity tests.—Inocula were prepared by suspending 10^7 cells/ml in SDW from 24-hr TCZ or YDC cultures. Onion leaves were inoculated at various stages of maturity by spraying noninjured leaves with a bacterial suspension, by spraying gently-rubbed, Carborundum-dusted leaves, or by infiltrating the suspension into leaf mesophyll with a tuberculin syringe fitted with a 0.4572-mm-diameter (26-gauge) needle. Plants were incubated at 25 and 30 C for 4-48 hr periods in moist chambers or under intermittent mist with an alternating cycle (30 sec on and 3 min off). Then the plants were placed on the greenhouse bench and observed daily for symptom development. Sliced and whole onion bulbs were inoculated by placing 0.05 ml of the bacterial suspension on outer or inner scales, with and without additional prick wounds.

Serology.—Immunizing antigens were prepared with

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three bulb-onion strains of *Xanthomonas* from Molokai (strains A88-3, A255-4, and A30-2a), *X. campestris* from cabbage (strain A249-1), *X. vesicatoria* from tomato (A135-1), *X. phaseoli* from snap bean (A127-2), *Xanthomonas* sp. from ti [*Cordyline terminalis* (L.) Kunth] (A273), *Xanthomonas* sp. from panax (*Polyscias guifoylei* Baily) (G715). All strains were rechecked for purity in culture and for pathogenicity on their homologous hosts. Strains were grown on yeast-glycerol agar (YGA) slants at 28 C to reduce polysaccharide production. The formulation for YGA is: 5.0 g yeast extract, 1.0 g K_2HPO_4, 0.5 $MgSO_4$, 20 g glycerol, and 20 g agar (Difco) per liter. Harvested cells were washed by suspending them in 0.85% saline and centrifugation at 7,710 *g* for 10 min. The pellet was resuspended in 0.5% formalin-saline, centrifuged, resuspended in saline, and placed in a 60 C water bath for 1 hr. Cells were washed twice with saline and resuspended in 0.3% formalin-saline to $A_{600} = 0.6$. Only G715 was adjusted to $A_{600} = 0.36$ because of its unusual toxicity to rabbits. Following a preimmune bleeding, the immunizing antigen was injected intravenously on day 1 (0.5 ml), day 3 (1.0 ml), day 6 (2.0 ml), and day 9 (2.0 ml). If the agglutination titer was less than 640 on day 11, two additional 3.0-ml injections were given on days 14 and 18. When the titer exceeded 640 the serum was collected. For agglutination tests, cells of 39 *Xanthomonas* strains were grown for 4 days, formalin-killed, heat inactivated at 60 C, washed, and resuspended to $A_{600} = 0.6$. Agglutination titers were determined with a microtiter kit obtained from Flow Laboratories, Inglewood, CA 90302.

Cross-reactivity of immune sera was determined for heterologous and homologous *Xanthomonas* strains. All titers were determined in duplicate.

Preparation of homologous antisera.—A pellet of centrifuged cells of an heterologous isolate was resuspended in a 1:30 dilution of immune sera containing (1:10,000) merthiolate, and incubated at 28 C for 2 hr. The suspension was centrifuged (7,710 *g*) for 15 min and the supernatant liquid was treated successively with each of the other heterologous strains. The final supernatant then was tested for agglutination with homologous and heterologous antigens. Adsorbed, homologous antisera then were tested for agglutination against 39 strains from bulb onions from the islands of Maui and Molokai. All strains also were tested for cross-reactions by the

Fig. 1. Field symptoms of bacterial blight caused in onions by *Xanthomonas* sp. showing tip dieback, premature death of outer leaves, and stunting.

Ouchterlony double-diffusion technique (10) with adsorbed and nonadsorbed antisera of onion strain A88-3 in the center well.

RESULTS

Isolation of the pathogen.—Typical *Xanthomonas* sp. colonies were recovered from 125 individual plants from onion-growing areas in Molokai and Maui during 1975-1977. No *Pseudomonas cepacia* was recovered, although presence of this widely spread onion pathogen was suspected. *Xanthomonas* was associated with watersoaked margins of leaf lesions (Fig. 2), as well as tip dieback and extensive blighting of outer leaves. Thus, the latter term is more descriptive of the overall effect on the plant.

On Molokai, the pathogen was recovered from Granex 33 onions grown on cleared brushland, planted to onion for the first time. Onions affected by the disease came from three separate seed sources, two from California and one from Texas. Despite numerous attempts to isolate the pathogen from seed with a selective medium, *Xanthomonas* sp. was not recovered. Nevertheless, when samples of both seed lots were planted in an experimental plot in a virgin area of Oahu, small white flecks appeared on the leaves from which *Xanthomonas* sp. could be readily isolated. On Maui, the pathogen was isolated from onion leaves of five separate farms. At two of these farms, purple blotch caused by *Alternaria porri* also was present; and at one farm, *Botrytis squamosa* was present in a nearby seedbed. Neither fungal disease occurred on Molokai. *Alternaria tenuis* and *Stemphylium* sp. often were present on leaf surfaces of healthy and diseased plants, but when reinoculated to susceptible hosts these fungi did not produce symptoms. Since fungi were not recovered consistently from typical lesions, and since the disease could be reproduced without a fungal association, their role was considered negligible.

Fig. 2-(a, b). Symptoms of bacterial blight of onion caused by *Xanthomonas* sp. **a)** Dew formation on upper leaf blade with watersoaked tissue beneath droplets, showing suspected passage of bacteria to leaf mesophyll. **b)** Lenticular lesion with watersoaked margins from which *Xanthomonas* sp. was isolated.

Identification of the pathogen.—In physiological and biochemical tests, all onion strains possessed the diagnostic features of the genus *Xanthomonas*. Cells were single, nonspore-forming, Gram-negative, straight rods, motile by a single flagellum, and produced a yellow pigment (on YDC) which was extractable in methanol but not in water. Metabolism was oxidative, not fermentative. All strains were oxidase-negative, catalase-positive, and did not reduce nitrates, or produce acetoin or indole. Asparagine was not utilized and growth was inhibited below 5 C and above 40 C. Sodium hippurate was not hydrolyzed in five days on Dye's YS broth (4). Variable results were obtained on YS broth containing peptone and glucose (4). Additional diagnostic features all corresponded to the *X. campestris* group (2). When four strains of *Xanthomonas* sp. from onion were compared with 22 other *Xanthomonas* strains from eight nomenspecies for utilization of 122 carbon sources, onion strains showed greatest similarity to two *X. dieffenbachiae,* three *Xanthomonas* strains from panax, and three from ti. Percent similarities were 87-98, 89-95, and 91-97%, respectively. The four onion strains showed 90-100% similarity with each other, 86-90% similarity with *X. phaseoli* and 74-92% similarity with 12 *Xanthomonas* sp. strains from orchid, ivy, banana, and vegetable crops.

Pathogenicity tests.—When Carborundum or needle injections were used to inoculate the plants, characteristic symptoms were produced. Small (0.5-mm diameter) yellowish-white lesions appeared 72 hr after inoculation and enlarged to lenticular watersoaked areas as the disease progressed. Chlorotic streaks often extended the entire length of the blade, and the leaf folded where lesions weakened it primarily on one side. Tip dieback also caused leaves to fold over at the necrotic margin. In controlled humidity chambers, control plants developed chlorotic streaks and tip dieback at 25 and 30 C. This could have been confused with disease symptoms except that no watersoaking or elongation of lenticular lesions occurred. To avoid this physiological stress response, misting was the preferred method of humidifying plants during incubation.

The effect of incubation time under mist on symptom development is shown in Table 1. Plants incubated for 4-8 hr developed large watersoaked lesions 13 days after inoculation, but those incubated 24 hr or more showed the pathogenic response in 3-8 days. Necrotic flecks and chlorosis characteristic of stress or a hypersensitive

response developed on plants inoculated with *X. phaseoli* (strain A127-2) which was isolated from bean plants in a nearby field, but this strain did not produce the typical watersoaked, lenticular lesions. No disease symptoms were produced by yellow-pigmented *Erwinia herbicola* commonly found on onion leaves.

The onion strains caused typical symptoms on bulb onion cultivars of *Allium cepa,* and on *A. fistulosum,* a green onion. Fifteen bulb onion cultivars including Granex 33 and Texas Grano 502 (yellow), Robust hybrid (red), and White Alamo hybrid (white) all were found to be susceptible although some degree of tolerance by the red and white cultivars was observed. Onion strains affected only the leaves; neither these nor *E. herbicola* caused perceptible damage to bulbs even when bulbs were sliced or pricked to allow entry of heavy inoculum (1×10^8 cells/ml). In contrast, *E. carotovora* rotted bulbs within 48 hr but leaves were not affected.

In cross inoculations onto alternate hosts, onion strains incited a hypersensitive response on bean, tobacco, and ivy. On bean, necrotic lesions with reddish margins formed in 48 hr and later became necrotic, with a shot-hole effect. On ivy, large black patches formed but in no case did watersoaking occur or did the necrosis spread beyond the inoculated area. *Xanthomonas* strains from bean, cabbage, strawberry, ivy, *Dieffenbachia,* and panax did not cause watersoaking on onion but did cause typical pathogenic symptoms on their homologous hosts.

Serology.—In agglutination tests, nonadsorbed sera of onion strains A88-3, A30-2a, and A255-4 cross reacted with heterologous *Xanthomonas* strains from cabbage (A249-1), tomato (A135-1), snap bean (A127-2), panax (G715), and ti (A273). Affinity patterns in agglutination tests showed closer relationships with *Xanthomonas* sp. from panax and ti than from bean, tomato, or cabbage. Adsorbed sera of the three onion strains agglutinated inactivated suspensions of all 39 onion strains from Maui and Molokai, but did not react with the heterologous antigens. In double-diffusion precipitin tests, all onion strains formed single bands with adsorbed antiserum of onion isolate A88-3, showing that onion strains were serologically related. Heterologous antigens formed no bands with adsorbed or nonadsorbed antisera of the onion strain A88-3, which showed that the heterologous strains were serologically distinct.

DISCUSSION

Tipburn and leaf dieback of onion resulting in undersized bulbs have been recurrent problems on the island of Maui for onion production at low elevations. Since the causes were attributed to windburn, thrips damage, excess nitrogen fertilization, and/or associated with purple blotch and Botrytis leaf blight, the bacterial nature of the problem was overlooked. However, when leaf blight became severe on the isolated island of Molokai, where the two fungal diseases were not present, the association of bacteria with disease symptoms was more carefully scrutinized. On Molokai, symptoms occurred in the absence of thrips, under a variety of fertilization regimes, and occasionally was more severe in 6.1-m (20-ft) strips adjacent to windbreaks than in the remainder of the field. Thus, as other factors were ruled out as primary causes, and a *Xanthomonas* sp. continually was associated with symptoms which could be reproduced in the greenhouse in the absence of wind damage, the bacterial nature of the problem was clearly defined. Wind damage and sandblast injury may facilitate entry of the pathogen in nature, but these are not indispensable for infection.

Bacteria probably are splashed from soil and infected plants onto leaf surfaces by sprinkler irrigation. Multiplication could occur in dew droplets which usually form on one side of the leaf as do the necrotic flecks and watersoaked lesions from which *Xanthomonas* was frequently isolated (Fig. 2-a). In the early morning, onion leaf tissue immediately beneath these droplets often was watersoaked. With alternating cycles of dew formation and sprinkler irrigation, pockets of disease could be established from a localized infection site and then could spread rapidly throughout the field. Movement of the pathogen in successive crops during the last 3 yr has followed this pattern.

The failure to recover *Xanthomonas* from onion seed does not preclude this as an inoculum source in Hawaii. *Xanthomonas* sp. is not known to survive for long periods as a free-living organism in soil (12), and all recognized *Xanthomonas* spp. so far have been found only in association with plants or plant materials (2). Because the disease was found in onion fields established in cleared brushland of Molokai, seed is implicated as the most likely source of inoculum.

Only one previous record of a similar disease of onion has been found (14). A bacterial leaf streak was observed in 1948 on Sweet Spanish onions in Colorado, and the causal organism was named *Xanthomonas striaformans*. Although symptom expression and disease development were similar, the physiological description of the pathogen differed in 8 of 18 characters, among these,

TABLE 1. Effect of incubation of onion plants on symptom development following inoculation of leaves with selected strains of bacteria[a]

Isolate and Host	Incubation period under mist at 25 C				
	4 hr	8 hr	24 hr	36 hr	48 hr
Xanthomonas sp. A88-3 (onion)	±[b]	±	+	+	+
Xanthomonas sp. A30-2a (onion)	±	±	+	+	+
X. phaseoli A127-2 (bean)	CS	CS	CS/NF	CS/NF	CS/NF
Erwinia herbicola A110-2 (onion)	−	−	−	−	−
Control (distilled water)	−	−	−	−	−

[a]After incubation plants were placed on the greenhouse bench. Symptoms were recorded on the eighth day after inoculation.
[b]Symbols and abbreviations: ± = necrotic flecks at 8 days which became watersoaked after 12 days; + = all characteristic symptoms (watersoaked lesions, streak, tipburn); CS = chlorotic streaks; NF = necrotic flecks; and − = no reaction.

production of nitrites from nitrates, weak hydrolysis of starch, and a "facultative aerobiosis" which is atypical of *Xanthomonas* spp. Since no cultures are available for further testing and the disease has not reoccurred in Colorado (L. E. Dickens, *personal communication*) the causal agents cannot be compared.

Since the *Xanthomonas* onion strains from Hawaii possessed all diagnostic features of the *X. campestris* group described in Bergey's Manual (2) and differed from the four other taxospecies, it could logically be included in the first group. However, because of its serological and pathological differences from *X. campestris* and other *Xanthomonas* spp. within the group, confusion will arise if it is not distinguished as a separate nomenspecies. We intend to do this in a separate publication.

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