Stalk and Leaf Necrosis of Onion Caused by *Erwinia herbicola*

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ABSTRACT

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A new foliar disease of onion (*Allium cepa*) in the Cape Province of South Africa was characterized by rapid necrosis of seed stalks and leaves. A bacterium isolated from diseased stalks was identified as *Erwinia herbicola*. Characteristic symptoms of the disease developed within 3–10 days when the organism was inoculated by needle injection into healthy flower stalks. The bacterium was isolated from artificially inoculated plants. This is the first record of *E. herbicola* as a pathogen of onion.

Onion (*Allium cepa* L.) is cultivated extensively for the production of seed in the semiarid Little Karoo of the southern Cape Province of South Africa. During the summer of 1977–1978, a disease of unknown etiology was noted on the cultivar Granex in a field of a major seed producer in the area. Disease loss on one 12-ha seed field exceeded $30,000 (U.S.).

The disease was characterized by rapid necrosis of seed stalks (Fig. 1A). Weakened stalks often failed to support seed heads and then collapsed. Necrosis was accompanied by breakdown of chlorophyll. Leaves of the plants were also affected, but stalk necrosis was considered a more reliable symptom of the disease. Diseased stalks eventually dried out or were decayed by secondary organisms, and seeds failed to develop.

Isolations from diseased stalks consistently yielded yellow pigmented, Gram-negative, peritrichously flagellated, rod-shaped bacteria. No pathogen with these properties is known to be associated with any disease of onion. This paper establishes the identity of the bacterial pathogen responsible for this stalk and leaf necrosis of onion.

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MATERIALS AND METHODS

Isolation. Diseased seed stalks were slit open longitudinally near the advancing margin of the necrotic area. Pieces of tissue 3 mm^3 were excised from the exposed inner surface, without piercing the epidermis, and crushed in 0.5 ml of sterile distilled water. Loopfuls of suspension were streaked on Difco nutrient agar (NA). Only bacteria developed and pure cultures were obtained from repeated subculturing on NA. Incubation was at 26 C.

Bacterial strains. Initial tests indicated that isolates from diseased stalks were identical in morphological, cultural, and biochemical properties. It was suspected that these isolates belonged to a single strain (coded SUH). This was confirmed in the present investigation, and no reference will be made to individual isolates of strain SUH. The strain was compared with *Erwinia herbicola* (Löhnis) Dye type strain NCPPB 2971 and strain PDDCC 787.

Identification. All strains were stained for flagella by the silver plating method (9) and by Hucker's modification of the Gram stain (11). Colony characteristics and pigmentation were determined on plates and slants of NA, modified yeast extract-dextrose-calcium carbonate agar (YDCA) (4), glucose-yeast extract-calcium carbonate agar (4), and nutrient skim milk agar (6). The capacity for mucoid growth was investigated on sucrose-nutrient agar (SNA) (4).

Biochemical tests included pectate degradation (12), oxidation of gluconate (10), oxidase (7), phenylalanine deaminase (5), the methyl red test, and indole production (6). Other tests were done as described by Dye (4). Acid production in media containing 1% of an organic

compound, 1% Difco peptone, and bromcresol purple indicator was determined after 7 days (4). The 21 compounds listed in Table 8.18 of Bergey's manual (2) were used. In all tests, inoculated media were incubated at 26 C unless indicated otherwise.

Pathogenicity tests. Fieldgrown onions (cultivar Caledon Globe) were inoculated in early summer when the bracts of the spathes started splitting during development of the first flowers. Inocula of SUH, NCPPB 2971, and PDDCC 787 strains were prepared by suspending 24-hr YDCA cultures in sterile distilled water at 10^4, 10^6, and 10^8 cells per milliliter. One-milliliter volumes of each cell suspension were injected into the green tissue and lacunar cavities of flower stalks, midway between the soil surface and the flower head, with a syringe fitted with an 18-gauge needle. Stalks of control plants were each injected with 1 ml of sterile distilled water.

RESULTS

Comparison of the bacterial strains. Strain SUH and two authentic *E. herbicola* strains were Gram-negative, facultatively anaerobic, rod-shaped

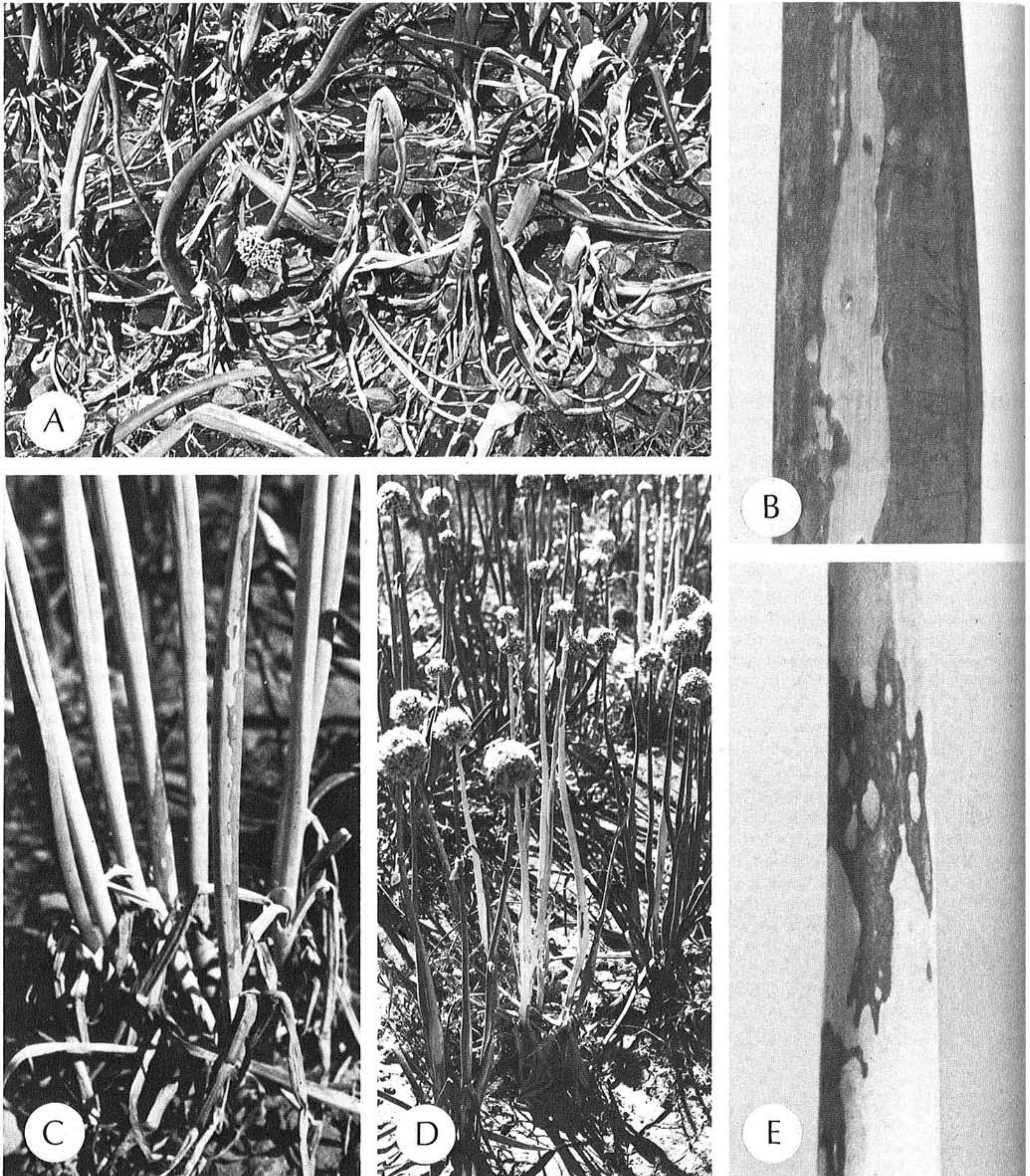

Fig. 1. Stalk and leaf necrosis of onion caused by *Erwinia herbicola*: **(A)** Naturally infected plants with severe disease. **(B–E)** Plants inoculated by injecting *E. herbicola* strain SUH into flower stalks. **(B)** Necrotic area around the point of inoculation. **(C and D)** Vertical spread of chlorotic areas. **(E)** Green "islands" of healthy tissue in necrotic area.

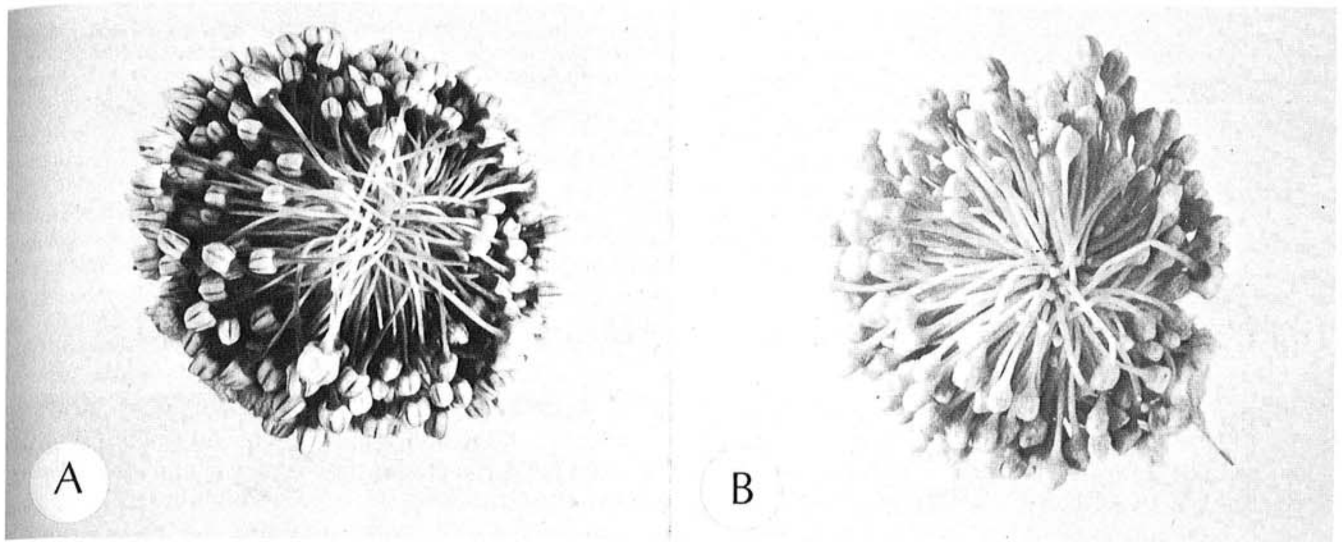

Fig. 2. Symptoms on flower heads of onion after inoculation with *Erwinia herbicola* strain SUH: **(A)** Chlorosis of pedicels followed by **(B)** chlorosis of the entire flower head.

bacteria, motile by peritrichous flagella. Colonies were yellow and similar on each growth medium. Mucoid growth was abundant on SNA.

The strains produced H_2S, reducing compounds from sucrose, acetoin, phenylalanine deaminase, and catalase; reduced nitrate to nitrite; liquefied gelatin at 22 C; and oxidized gluconate. They grew at 37 C but not at 40 C. The three strains fermented glucose, but lactose was fermented only by strain SUH.

None of the strains required growth factors, hydrolyzed casein or starch, decarboxylated arginine, glutamic acid, lysine, or ornithine; none produced gas from glucose, degraded potato tissue or pectate; and none formed oxidase or indole, gave a positive result in the methyl red test, or utilized benzoate, propionate, or oxalate.

The three strains produced acid from arabinose, mannitol, salicin, xylose, maltose, esculin, glycerol, mannose, rhamnose, and ribose. Acid was not produced from α-methyl glucoside, raffinose, dulcitol, inositol, melezitose, adonitol, or dextrin. Strain SUH produced acid from melibiose and cellobiose. Strains NCPPB 2971 and PDDCC 787 did not utilize these compounds. Only strain PDDCC 787 produced acid from sorbitol.

Pathogenicity tests. Strain SUH induced symptoms on the plants. Control plants and those inoculated with strains NCPPB 2971 and PDDCC 787 remained symptomless.

The first symptoms usually appeared after 3–4 days after inoculation. Although most of the inoculum was deposited in the lacunar cavity, lens-shaped, slightly sunken, necrotic lesions developed around the point of inoculation (Fig. 1B). Necrosis was accompanied by chlorosis. Chlorotic lesions extended rapidly (Fig. 1C), and most of the diseased stalks contained little chlorophyll after 10 days (Fig. 1D). However, green "islands" with sharply defined borders remained intact inside the necrotic areas on most stalks (Fig. 1E).

Necrosis was not confined to inoculated stalks and often spread to leaves and other stalks on the same plant. Symptoms also spread from the stalks to flower heads no longer enclosed by the spathe. Chlorosis of the pedicels (Fig. 2A) preceded chlorosis of the entire flower head (Fig. 2B). Little or no necrosis was evident in the green tissue enclosed by the bracts of the spathe or by the sheaths at the base of green leaves.

Ten days after inoculation, 40, 37, and 7 flower stalks (of 40) injected with 10^8, 10^6, and 10^4 cells, respectively, showed symptoms. The pathogen was isolated from diseased flower stalks, leaves, and pedicels of inoculated plants. Selected comparative bacteriologic tests confirmed that strain SUH had been reisolated.

DISCUSSION

The pathogen associated with stalk and leaf necrosis of onion is believed to be a strain of *E. herbicola*. It closely resembles two reference strains of the species and, as far as can be determined, differs only in respect of the utilization of carbohydrates. Although *E. herbicola* is generally regarded as a saprophyte, several different strains cause galls on Japanese wisteria (*Wistaria floribunda* (Willd.) DC) (13), internal necrosis of cotton bolls (*Gossypium hirsutum* L.) (1), pink disease of pineapple (*Ananas comosus* (L.) Merr.) (3), and purple stain of papaya (*Carica papaya* L.) (8). The addition of stalk and leaf necrosis of onion to this list emphasizes the diversity of plant diseases initiated by the organism.

Symptoms induced by *E. herbicola* strain SUH on test plants were similar to those observed in the onion field where the disease was first recorded. The most prominent features were the rapid breakdown of chlorophyll, the lack of breakdown where green tissue was covered by leaf sheaths or bracts, and the clear "islands" that remained in the necrotic areas. We are now investigating the physiologic basis of symptom expression.

Stalk and leaf necrosis has not been observed in the field since it was first recorded. We suspect that a combination of favorable environmental conditions and a high population of the pathogen resulted in the original disease outbreak. This is in part supported by our finding that only 17.5% of the 40 flower stalks became diseased when injected with 10^4 cells of the pathogen, compared with 100% of those injected with 10^8 cells.

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