Occurrence of *Pseudomonas syringae* on Bean and Soybean in Kenya

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ABSTRACT

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A bacterial disease of unknown etiology affected the foliage of bean (*Phaseolus vulgaris*) and soybean (*Glycine max*) plants in the highlands of Kenya. The bean and soybean bacterial strains were indistinguishable in cultural, morphologic, and physiologic properties and in their pathogenicity to various legume species. In greenhouse inoculation tests, bean and soybean bacterial strains produced brown, angular lesions on the foliage of bean, cowpea (*Vigna unguiculata*), lima bean (*P. lunatus*), soybean, and tepary bean (*P. acutifolius*) in 3–4 days. Symptoms on bean leaves were identical to those incited by *Pseudomonas syringae*, the causal organism of bacterial brown spot of bean. Both bacterial strains were seedborne in bean. Based on bacteriological tests, pathogenicity studies, and symptomatology, Kenyan bean and soybean bacterial strains are considered to be strains of *P. syringae*.

Several food legumes are grown in Kenya from the hot, humid areas near sea level up to the cool highlands at elevations above 2,500 m (1). Most crops are consumed locally, but the dry seeds or green pods of some bean (*Phaseolus vulgaris* L.) cultivars may be exported to Europe. Soybean (*Glycine max* (L.) Merr.) is being grown experimentally in Kenya because of its potential as a high protein oil crop.

In 1977, a bacterial disease of unknown etiology affected the foliage of snap beans and soybeans at two locations in the Kenyan highlands (>1,500 m). The symptoms were distinct from those of two other bacterial diseases that affect the foliage and pods of bean in Kenya; the known diseases are incited by the halo blight bacterium *Pseudomonas phaseolicola* (Burkh.) Dows. and the bacterial blight bacterium *Xanthomonas phaseoli* (E. F. Smith) Dows. (2,12).

This study was undertaken to identify the unknown bacterial pathogen. In this article, the nomenspecies of *Pseudomonas* and *Xanthomonas* species are used rather than the taxospecies of *P. syringae* Van Hall and *X. campestris* (Pammel) Dows., respectively (3).

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MATERIALS AND METHODS

Isolations were made from necrotic lesions on bean and soybean leaves on petri plates containing nutrient agar (NA), nutrient-5% sucrose agar, or yeast extract-dextrose-calcium carbonate agar (YDCA) (19). Cultures were maintained on YDCA slants or in distilled water at 4 C. Inoculum for pathogenicity tests was grown on NA or YDCA plates 48–72 hr at 22–24 C. Bacteria were washed off these plates with sterile distilled water, and the concentration was adjusted turbidimetrically to about 10^7 cells per milliliter.

A pathogenic strain of the bacterium from bean and another from soybean were included in all tests. These same strains were also sent to the Commonwealth Mycological Institute, England, for confirmation of identity and were given the following IMI numbers: B7924 (bean strain) and B7925 (soybean strain).

Selected physiologic and biochemical tests were performed with the strains according to the methods described by Lelliott et al (15). These included levan production, tyrosinase and oxidase activity, fluorescent pigment production, soft rotting of potato, presence of arginine dihydrolase, and hypersensitivity reaction on tobacco (*Nicotiana tabacum* L. 'White Burley'). Fluorescence on King's B medium was observed in ultraviolet light at 2537 Å. Gram staining was done according to methods outlined by Cowan and Steel (5). Electron micrographs were taken of bacterial cells that were negatively stained with 0.2% sodium phosphotungstic acid, pH 6.3.

The pathogenicity of both bacterial strains was tested by using a Paasche airbrush at about 1.05 kg/cm^2 (15 psi) to atomize a bacterial suspension (about 10^7 cells per milliliter) on the lower surface of the first set of trifoliolate leaves of test plants (18). After inoculation, plants were incubated in a humid atmosphere 48–72 hr at 20–24 C. Plants used in the inoculation studies were bean cultivars Long Tom and Black Turtle Soup, tepary bean (*P. acutifolius* A. Gray 'Tepary 11'), lima bean (*P. lunatus* L. 'Jackson Wonder'), soybean cv. Congo and HLS 541, and cowpea (*Vigna unguiculata* (L.) Walp. subsp. *unguiculata* 'California No. 5').

For evaluation of seed transmission in bean, developing pods of Black Turtle Soup and Long Tom bean were inoculated with the bean and soybean bacterial strains either by injuring the epidermis of pods over developing seeds with a sterile needle dipped into bacterial inoculum or by injecting a bacterial suspension (about 10^7 cells per milliliter) into the cavity of a pod with a syringe. Seeds harvested from inoculated and noninoculated pods were tested for infection either by plating them on NA after surface sterilization in 1% sodium hypochlorite for 1 min and rinsing in sterile distilled water or by planting them in vermiculite according to the procedure outlined by Grogan and Kimble (8).

RESULTS

Symptoms and distribution of disease. In February 1977, diseased foliage from a commercial snapbean planting (cv. Monel) near Nairobi was sent to the National Agricultural Laboratory in Kenya for identification of the causal agent. The necrotic lesions were angular with brown centers and ranged from 0.5 to 5.0 mm or larger in diameter. Most lesions were surrounded by a narrow yellow border. No pod lesions were observed. Disease incidence and severity could not be determined in this planting. Diseased soybean foliage (cv. Congo) was collected in November 1977 from plants in a date-of-planting trial at the Nyanza Agricultural Research Station, Kisii. Disease prevalence and severity were high (necrotic lesions were on most leaves in >90% of the plants). The disease was observed again in another date-of-planting trial with Congo soybean at the Kisii station in March 1978. The necrotic lesions on naturally infected soybean

leaves were similar in size and shape to those on bean leaves, except that the brown, angular lesions were surrounded by a light green halo (Fig. 1). Some soybean leaves had a shot-hole appearance when the centers of older lesions became torn (Fig. 1). Isolation from necrotic lesions on bean and soybean leaves onto NA yielded white bacterial colonies, similar to those of pseudomonads; cells from these colonies were pathogenic to the foliage of both plant species.

Pathogenicity studies. The host range and symptoms produced by pseudomonadlike bean and soybean strains were identical in greenhouse inoculation studies. Lesions with necrotic centers developed on inoculated trifoliolate leaves of bean (Fig. 2), lima bean, tepary bean, cowpea, and soybean in 3–4 days. Necrotic lesions often were observed on cowpea in 24–48 hr, particularly with higher levels of inoculum (10^8 to 10^9 cells per milliliter). The angular lesions on bean, which were not watersoaked, frequently increased in size and coalesced to cause deformation of the inoculated leaflet (Fig. 2). A narrow yellow zone often surrounded the brown lesions on inoculated bean foliage. A greenish halo usually surrounded the necrotic lesions on soybean leaves. Vein necrosis occurred on inoculated leaves of bean, lima bean, and tepary bean. No systemic symptoms were observed on any of the test plants. Symptoms developing on the foliage of bean and soybean inoculated in the greenhouse with both bacterial strains were similar to symptoms on field-infected plants.

Biochemical and physiologic tests. Bean and soybean bacterial strains were indistinguishable in cultural, morphologic, and physiologic properties. Both had the following bacteriologic properties that placed them in the genus *Pseudomonas*: they were aerobic, motile, gram-negative rods with one to several polar flagella; they produced a diffusible green, fluorescent pigment on King's B medium; they were oxidase negative but positive for tyrosinase activity and levan formation; they were negative for arginine dihydrolase activity; they did not reduce nitrate to nitrite; they produced acid from sucrose; they hydrolyzed aesculin and liquefied gelatin; they were unable to rot potato slices; and they induced a hypersensitive reaction in 24–48 hr when infiltrated into tobacco leaves.

Seed transmission. Reddish brown sunken lesions 0.5–6.0 mm or larger in diameter developed in 7–14 days on pods of Black Turtle Soup and Long Tom bean, on which the epidermis over the site of a developing seed had been injured with a needle dipped in a bacterial suspension; more than 25% of the seeds were discolored. Young pods inoculated by injecting a bacterial suspension into the pod cavity frequently became discolored and shriveled. More than 90% of the seeds from inoculated pods were discolored and unusually small. Bean and soybean bacterial strains were recovered from more than 80% of the discolored seeds collected from pods inoculated by either method and surface sterilized before plating on NA. When seeds from inoculated pods were germinated in vermiculite, the bacterial pathogen was isolated from $<10\%$ of the seedlings. No pathogenic bacteria were isolated from seeds in the healthy control series.

DISCUSSION

Pseudomonad strains from bean and soybean could not be differentiated by biochemical and physiologic tests, including the LOPAT test of Lelliott et al (15). The two Kenyan strains belong to group Ia or IA of the classification schemes developed by Lelliott et al (15) or Misaghi and Grogan (16), respectively. The host range and symptoms elicited by strains from bean and soybean were identical and similar to those produced by strains of *P. syringae* that cause bacterial brown spot of bean in the United States (11,17,18) and Australia (9). Based on results of laboratory tests, pathogenicity studies, and symptomatology, the two Kenyan strains are identified as *P. syringae*. The identity was also confirmed as *P. syringae* by the Commonwealth Mycological Institute, where the Kenyan bacterial pathogens were compared with many cultures of *P. phaseolicola* and *P. glycinea* and found to be identical to each other but distinct from the other two pseudomonads.

Fig. 1. Leaves of Congo soybean naturally infected by *Pseudomonas syringae*. Note shot holes. Necrotic lesions are surrounded by a light green halo.

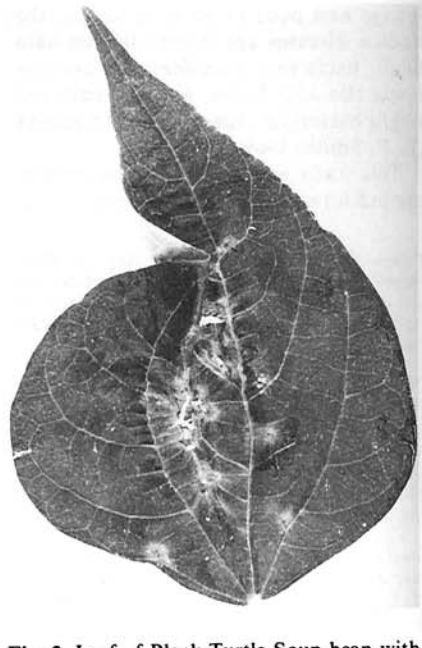

Fig. 2. Leaf of Black Turtle Soup bean with necrotic, angular lesions caused by the Kenyan bean isolate of *Pseudomonas syringae*. Brown lesions are surrounded by a narrow yellow border. Coalescing of lesions has resulted in deformation of the leaf.

This appears to be the first report of *P. syringae* as a foliar pathogen of bean and soybean in East Africa (Kenya, Tanzania, and Uganda). Many strains of *P. syringae* have been identified that vary greatly in their host ranges (10). Some isolates of the pathogen also are reported to infect soybean (4,6,20). The disease was particularly severe and widespread on soybeans at one location in the Kenyan highlands. In our greenhouse inoculation studies, the two strains of *P. syringae* produced typical bacterial brown spot symptoms on bean (9,11,17,18). These two isolates also produced symptoms on cowpea similar to those described by Lai and Hass (13).

In greenhouse studies, we demonstrated that both the bean and soybean isolates of *P. syringae* were seedborne in bean. It is possible that the bean and soybean pathogens were introduced into Kenya on seeds of bean, soybean, or other food or forage legumes that were imported for research or commercial use. It is not known whether seed transmission of *P. syringae* is important in spread or survival of the bacterium in Kenya. Besides its survival and transmission in seeds, inoculum of the bacterial brown spot pathogen may also survive adverse periods as an epiphyte on foliage, as demonstrated by Leben et al (14) for bean and Ercolani et al (7) for hairy vetch (*Vicia villosa*).

In the United States and Australia, bacterial brown spot occasionally is an important disease of green beans (9,11,17). This disease could become economically important in Kenya if it is widely distributed in commercial green bean plantings, the pods of which are exported to Europe. A high incidence of field infection by the bacterium, with pod distortion, blemishes, or both, could reduce prices or cause rejection of entire consignments of green pods.

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