AFR
633.33
K13

PN-AAH-2

Vol. 63, No. 4--PLANT DISEASE REPORTER--April 1979

TWO BACTERIAL DISEASES OF COWPEA IN EAST AFRICA

Walter J. Kaiser and A. H. Ramos

Research Plant Pathologist, Kenya Agriculture Research Institute, P.O. Box 30148, Nairobi, Kenya, and Bacteriologist, National Agricultural Laboratories, P.O. Box 14733, Nairobi, Kenya.

Present address of senior author: Regional Plant Introduction Station, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, 59 Johnson Hall, Washington State University, Pullman 99164.

Contribution of the Kenya Agriculture Research Institute (KARI), Muguga, Kenya; the National Agricultural Laboratories, Ministry of Agriculture, Nairobi, Kenya; the U.S. Agency for International Development (Food Crops Research Project 618-11-110-657); and the Animal Plant Health Inspection Service of the U.S. Department of Agriculture.

The authors express their appreciation to Loise M. Muriithi, KARI, Muguga, for her competent technical assistance; to E. J. Guthrie, KARI, Muguga, for photographs of disease symptoms; to M. Chimtawi, International Centre for Insect Physiology and Ecology, Nairobi for taking electron micrographs of bacterial cells; and to T. Sengooba, Kawanda Research Station, Kampala, Uganda, for providing leaves of beans naturally infected with Xanthomonas phaseoli var. fuscans.

Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

ABSTRACT

Cowpeas (Vigna unguiculata) in East Africa were infected by two seedborne xanthomonads that produced distinct and different disease symptoms on the foliage. The bacteria causing cowpea bacterial blight (CBB) and cowpea bacterial pustule (CBP) were indistinguishable in cultural, morphological, and physiological properties, and in their pathogenicity to different legume species. Both infected several Phaseolus and Vigna species. Isolates of CBB and CBP could be differentiated only by the symptoms that they produced on cowpea foliage. Both bacteria are considered to be strains of Xanthomonas phaseoli f. sp. vignicola (a member of the X. campestris group). East African isolates of CBB and CBP could be differentiated from the bacterium causing fuscous blight of bean (X. phaseoli var. fuscans (Xpf)) by their pathogenicity to bean and cowpea. The CBB and CBP pathogens were pathogenic to both bean and cowpea, whereas Xpf was pathogenic to bean only.

Plant Dis. Reptr. 63: 304-308.

Additional key words: seedborne disease.

Cowpea (Vigna unguiculata) is one of several legumes that are cultivated as a human food crop in East Africa, particularly in the warmer, lower elevation areas around Lake Victoria and along the East African Coast (1). Numerous diseases and pests affect V. unguiculata, contributing significantly to their low yields and poor quality wherever they are grown in Africa (1,14,16).

Of the diseases affecting cowpeas in Africa, least appears to be known of those caused by bacteria (16). In East Africa, the authors have observed cowpeas infected by two bacterial pathogens that produced distinct symptoms on the foliage. This paper describes the symptoms, bacteriological tests, and pathogenicity of these xanthomonads that cause cowpea bacterial blight (CBB) and cowpea bacterial pustule (CBP). These bacteria are also compared with an isolate of Xanthomonas phaseoli (E. F. Smith) Dowson var. fuscans (Burkh.) Starr & Burkh. (Xpf), which causes fuscous blight of bean (Phaseolus vulgaris). The nomenspecies of Xanthomonas spp. is used in this paper rather than the taxospecies, X. campestris (Pammel) Dowson (2).

MATERIALS AND METHODS

Cultures of the CBB pathogen were obtained from the foliage of diseased cowpea plants grown at the Agricultural Research Institute, Ilonga, Kilosa, Tanzania. Those of the CBP pathogen were isolated from diseased cowpea leaves collected at the Faculty of Agriculture and Forestry, University of Dar es Salaam, Morogoro, Tanzania; the Agricultural Research Institute, Mligano, Tanga, Tanzania; and the Coast Agricultural Research Station, Mtwapa,

Mombasa, Kenya. A culture of Xpf was obtained from diseased bean leaves from the Kawanda Research Station, Kampala, Uganda.

Diseased plant specimens were transported to the laboratory at Muguga, Kenya in clean plastic bags. For isolation of bacteria, small pieces of tissue from the advancing margins of lesions were placed in sterile distilled water for 15 min to 2 hr. A loopful of liquid was streaked on petri plates containing nutrient agar (NA) or yeast extract-dextrose-calcium carbonate agar (YDCA) (10). Cultures were maintained in distilled water or on YDCA slants at 4°C. Inoculum was prepared by streaking a loopful of bacterial suspension on NA or YDCA and incubating the slants for 48 hr at laboratory temperature. Bacteria were washed from the medium with sterile distilled water and diluted to about 10^8 cells/ml.

Seeds of test plants for inoculation studies were planted in steam-sterilized soil in 2-liter plastic pots. Young plants were inoculated by one of two methods: (i) atomizing a bacterial suspension on the lower surface of young expanding leaves with a Paasche air brush apparatus at about 1.05 kg/cm^2 (15 psi), or (ii) jabbing a sterile needle through a drop of inoculum placed in the axis of the first or second node. Inoculated plants were incubated in a humid atmosphere for 7-10 days at temperatures ranging from 25-30°C. Test plants used in the inoculation studies are listed in Table 1.

Selected bacteriological tests performed according to the methods of Lelliott, et al. (8) were: lipolysis of coconut oil, ability to induce potato rot, and oxidation/fermentation tests with bromothymol blue as a pH indicator. All other bacteriological tests, including gram staining, were done according to the methods outlined by Cowan and Steel (3). For electron microscopic studies, drops of liquid cultures grown in Kelman and Hrascha's medium (6) were allowed to dry slowly on collodion-coated grids, and bacterial cells were negatively stained with 2% sodium phosphotungstic acid, pH 6.3. Induction of hypersensitivity of infiltrated tobacco leaves (*Nicotiana tabacum* 'White Burley') was tested in the laboratory at 20-22°C by the method of Klement (7).

Table 1. Comparison of pathogenicity of East African isolates of *Xanthomonas* from bean and cowpea to stems of different plants species[a].

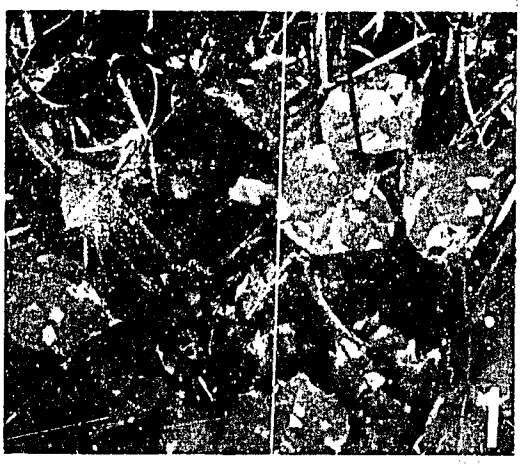
Host	Disease rating of isolate[b]		
	CBB	CBP	Xpf
Arachis hypogaea 'Natal Common'	2	2	1
Cajanus cajan 'T 21'	2	3	1
Dolichos lablab	3	3	1
Glycine max 'HLS 541'	2	3	1
Macroptilium lathyroides	3	4	3
Phaseolus acutifolius EAI 2874[c]	3	3	4
P. lunatus 'Jackson Wonder'	4	3	3
P. vulgaris 'Canadian Wonder'	4	5	9
P. vulgaris 'Light Red Kidney'	6	6	10
P. vulgaris 'Long Tom'	5	6	9
Pisum sativum 'Wisconsin Perfection'	3	7	3
Vigna aconitifolia EAI 2873	5	8	2
V. angularis EAI 5363	6	7	2
V. radiata	3	3	3
V. umbellata PI 247692	8	8	3
V. unguiculata 'California No. 5'	9	10	3
V. unguiculata 'Vita 1'	10	10	2
V. unguiculata 'Zipper Cream'	10	10	2

[a]The isolates were: CBB, cowpea bacterial blight-inducing isolate of *X. phaseoli* f. sp. *vignicola* (Xpv); CBP, cowpea bacterial pustule-inducing isolate of Xpv; and Xpf, bean fuscous bacterial blight-inducing isolate of *X. phaseoli* var. *fuscans*. [b]Disease rating scale: 1 = no infection, to 10 very severe response (development of stem cankers, followed by wilting and death of inoculated plants). Ratings of 2-9 represent responses intermediate between those rated 1 and 10. Disease ratings were recorded 12-15 days after inoculation and were the averages of 2-3 experiments with 3-6 plants/experiment. [c]Plant species with EAI numbers are plant accessions introduced by the Plant Quarantine Station, Muguga, Kenya, while those with PI numbers are from the U.S. Department of Agriculture.

Seed transmission of the two cowpea bacterial isolates was studied with California No. 5 cowpea seed that had been inoculated by (i) slightly injuring the epidermis of the pod over the site of a developing seed with a sterile needle dipped in a bacterial suspension, or (ii) injecting a bacterial suspension into the cavity of nearly mature pods. Seeds from inoculated and non-inoculated pods were planted in steam-sterilized soil.

RESULTS

Disease symptoms: The initial symptoms of CBB were small water-soaked spots on the foliage. Tissues surrounding these spots became necrotic and irregular in shape. Individual lesions varied considerably in size, ranging from 0.5-2 cm or larger (Fig. 1). Lesions were generally tan colored and surrounded by a yellow zone (0.1-0.5 cm wide). Lesions sometimes coalesced to encompass large portions of the leaf, which turned yellow and wilted. Cankers, particularly in artificially inoculated plants, developed on the stems of severely infected plants (Fig. 2). Initial symptoms of CBP on the underside of leaves were small, raised, water-soaked pustules that increased in size to about 0.1-0.4 cm, remaining more or less circular.



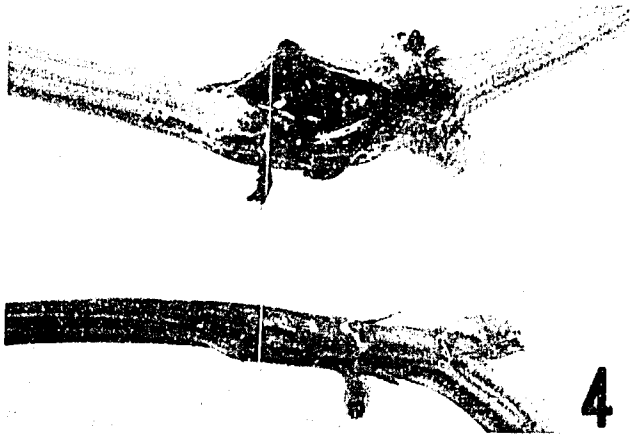


FIGURE 1. Cowpeas naturally infected by an isolate of Xanthomonas phaseoli f. sp. vignicola (Xpv) causing cowpea bacterial blight in East Africa.

FIGURE 2. Canker on the stem of a cowpea plant inoculated at the lower node with an isolate of Xpv causing cowpea bacterial blight.

FIGURE 3. Naturally infected cowpea leaf exhibiting symptoms of cowpea bacterial pustule.

FIGURE 4. Cankers on stems of Phaseolus vulgaris 'Red Kidney' (top) and Vigna umbellata (bottom) inoculated at first node with a cowpea bacterial pustule-inducing isolate of Xpv.

On the upper surface of the leaf, the centers of these lesions, which were surrounded by a yellow halo, became necrotic and sunken (Fig. 3). Symptoms of CBB and CBP on cowpea in East Africa were similar to those observed by Williams (16) on cowpea in Nigeria.

Occurrence of disease: In May 1975, the senior author visited several research stations in important cowpea-growing areas of Tanzania and Kenya. Symptoms of CBB were observed on cowpea plants in yield trials at the Agricultural Research Institute (ARI), Ilonga at Kilosa, Tanzania. The disease was widespread and serious on several cowpea lines. The original source of seeds of most lines was Nigeria. Symptoms of CBP were observed on cowpea lines in varietal plantings at the Faculty of Agriculture and Forestry, University of Dar es Salaam, Morogoro, Tanzania; the ARI, Mlingano at Tanga, Tanzania; and the Coast Agricultural Research Station, Mtwapa at Mombasa, Kenya. Seeds for the Morogoro and Mlingano cowpea plantings were obtained from the ARI, Ilonga, whereas those for the Mtwapa plantings had been imported directly from Nigeria. At the three locations where CBP was observed, many varieties were infected, but disease intensity was low.

Laboratory characterization of the bacteria: Isolates of the CBB and CBP pathogens were indistinguishable from one another in cultural, morphological, and physiological properties. Both had the following bacteriological properties, which places them in the genus Xanthomonas: they were aerobic, motile, gram-negative rods with a single monotrichous flagellum; they formed convex yellow colonies on nutrient agar in 3 days at 25°C; they oxidized galactose, mannitol, raffinose, sucrose, and xylose but not lactose; they utilized citric and succinic acids but not malic or tartaric acids, as sole sources of carbon; they produced amylase gelatinase but not β-glucosidase; they formed hydrogen sulfide but did not reduce nitrate to nitrite or produce ammonia; they were actively lipolytic; they were unable to rot potato slices; and they did not induce a hypersensitive reaction when infiltrated into tobacco leaves. The only bacteriological test that differentiated Xpf from the CBB and CBP pathogens was production by Xpf of a brown diffusible pigment on nutrient agar.

Seed transmission: The causal agents of CBB and CBP were transmitted in cowpea seeds harvested from artificially inoculated pods. Seeds harvested from inoculated pods frequently were discolored and shriveled. Pathogenic bacteria were reisolated from internal tissues of surface-sterilized seeds collected from pods inoculated with bacteria, but not from seeds of pods inoculated with sterile water. Seed transmission also was demonstrated in seeds from pods inoculated with both isolates when planted in steam-sterilized soil. Typical CBB and CBP symptoms developed in 10-14 days on the primary leaves of 5-10% of the germinating seedlings, and both pathogens could be reisolated from the lesions. Stem cankers developed occasionally in plants infected from seed, particularly with the CBB pathogen.

Pathogenicity studies: The organisms causing CBB and CBP were pathogenic to the foliage and stems of several legume species in greenhouse inoculation tests. Inoculation of stems (Table 1) proved to be a more reliable test of pathogenicity than inoculation of the foliage. Both isolates were moderately to highly pathogenic to bean (P. vulgaris) and all Vigna spp. except V. radiata. By contrast, Xpf was highly pathogenic to bean but only weakly pathogenic to all Vigna spp., including cowpea (Table 1). Cankers frequently developed at the site of inoculation on stems of Vigna spp. and beans inoculated with CBB and CBP (Fig. 4) and beans inoculated with Xpf. Inoculated plants often wilted and died when cankers increased in size and girdled the stem, as often happened when cowpeas were inocualated with the CBB and CBP pathogens and beans inoculated with Xpf.

DISCUSSION

Relatively little is known of the bacterial diseases that affect food legumes, including cowpeas, in Africa. In 1975, Williams (16) listed bacterial pustule and bacterial blight as two of the important diseases of cowpea in Nigeria. His description of CBP apparently is the first report of this disease in the literature; ours is the first report of both bacterial diseases of cowpea in East Africa. The seedborne nature of CBB in cowpea was confirmed by Shekhawat, et al. (12); we have demonstrated that the CBP pathogen is seedborne in cowpea. It appears that the incitants of CBB and CBP may have been introduced into East Africa in different consignments of cowpea seed that originated from Nigeria, where both diseases occur. This illustrates the ease with which potentially important pathogens may be introduced into new areas on contaminated seed unless special precautions are taken. The importance of clean (pathogen-free) seed in the transfer of cowpea germplasm for research and other purposes must be emphasized.

In East Africa, cowpeas are grown as a rainfed crop and generally are planted during the rainy season. In Nigeria, disease development and spread of CBB and CBP are favored by high

rainfall (16). If either or both of these bacterial diseases become more widespread and serious on cowpeas in East Africa, particularly in high rainfall areas, it may be necessary to implement a control program involving the use of clean seed, resistant varieties, or both. Sources of resistance to CBB and CBP in cowpea are known (9,13,16).

The bacteriological properties of East African isolates of CBB and CBP are within the range of variability exhibited by the members of the genus Xanthomonas (2,4,11). Isolates of the CBB and CBP pathogens from Kenya and Tanzania were indistinguishable in biochemical, morphological, and cultural characteristics. Their pathogenicity to different leguminous hosts was similar. They could be differentiated by the symptoms that formed on inoculated cowpea foliage, but not by those that formed on inoculated stems. We feel that these differences in symptom expression on cowpea are insufficient to warrant designating the bacteria inciting CBB and CBP as separate species.

Recent studies with pathogenic xanthomonads isolated from blighted beans in Puerto Rico have identified two Xanthomonas pathogenic types based on pathogenicity to bean and cowpea (5, 15). Isolates pathogenic to bean only were designated X. phaseoli (Xp) or X. phaseoli var. fuscans (Xpf), while those pathogenic to both hosts were called X. phaseoli f. sp. vignicola (Burkh.) Sabet (Xpv). East African isolates of CBB and CBP were similar in their pathogenicity to Puerto Rican isolates of Xpv. The Ugandan Xpf isolate was similar in its pathogenicity to Puerto Rican isolates of Xp and Xpf. From these results, we conclude that the pathogenic xanthomonads that cause bacterial blight and bacterial pustule of cowpea in East Africa are strains of the same bacterium, namely Xpv.

Literature Cited

1. BOCK, K. R. 1973. East African strains of cowpea aphid-borne mosaic virus. Ann. Appl. Biol. 74: 75-83.
2. BUCHANAN, R. E., and N. E. GIBBONS (Eds.). 1974. Bergey's manual of determinative bacteriology, 8th ed. Williams and Wilkins Co., Baltimore. 1,246 pp.
3. COWAN, S. T., and K. J. STEEL. 1965. Manual for the identification of medical bacteria. Cambridge Univ. Press, London. 217 pp.
4. DYE, D. W. 1962. The inadequacy of the usual determinative tests for the identification of Xanthomonas spp. N. Z. J. Sci. 5: 393-416.
5. KAISER, W. J., and N. G. VAKILI. 1978. Insect transmission of pathogenic xanthomonads to bean and cowpea in Puerto Rico. Phytopathology 68: 1057-1063.
6. KELMAN, A., and J. HRASCHA. 1973. The role of motility and aerotaxis in the selective increase of avirulent bacteria in still broth cultures of Pseudomonas solanacearum. J. Gen. Microbiol. 76: 177-188.
7. KLEMENT, Z. 1963. Rapid detection of the pathogenicity of phytopathogenic pseudomonads. Nature (Lond.) 199: 299-300.
8. LELLIOTT, R. A., E. BILLING, and A. C. HAYWARD. 1966. A determinative scheme for the fluorescent plant pathogenic pseudomonads. J. Appl. Bacteriol. 29: 470-489.
9. PATEL, P. N., and J. K. JINDAL. 1970. Studies on resistance to bacterial diseases in India. II. Resistance in cowpea to bacterial blight (canker) disease. Bull. Indian Phytopathol. Soc. 6: 28-34.
10. SAETTLER, A. W. 1971. Seedling injection as an aid in identifying bean blight bacteria. Plant Dis. Reptr. 55: 703-706.
11. SCHUSTER, M. L., and D. P. COYNE. 1977. Characterization and variation of Xanthomonas and Corynebacterium incited diseases of bean (Phaseolus vulgaris L.). Fitopatol. Bras. 2: 199-209.
12. SHEKHAWAT, G. S., P. N. PATEL, and S. RAJ. 1977. Histology of cowpea plant infected with Xanthomonas vignicola. Z. Pflanzenkr. Pflanzenschutz 84: 547-558.
13. SHERWIN, H. S., and C. L. LEFEBVRE. 1951. Reaction of cowpea varieties to bacterial canker. Plant Dis. Reptr. 35: 303-317.
14. SUMMERFIELD, R. J., P. A. HUXLEY, and W. STEELE. 1974. Cowpea (Vigna unguiculata (L.) Walp.). Field Crop Abstr. 27: 301-312.
15. VAKILI, N. G., W. J. KAISER, J. E. PÉREZ, and A. CORTÉS-MONLLOR. 1975. Bacterial blight of beans caused by two Xanthomonas pathogenic types from Puerto Rico. Phytopathology 65: 401-403.
16. WILLIAMS, R. J. 1975. Diseases of cowpea (Vigna unguiculata (L.) Walp.) in Nigeria. PANS (Pest Artic. News Summ.) 21: 253-267.