Detection of *Xanthomonas campestris* pv. *vignicola* in Southern Pea Seed

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ABSTRACT

Gitaitis, R. D., and Nilakhe, S. S. 1982. Detection of *Xanthomonas campestris* pv. *vignicola* in southern pea seed. Plant Disease 66:20-22.

A method was devised for detecting *Xanthomonas campestris* pv. *vignicola* in southern pea (*Vigna unguiculata*) seeds. Samples of 100 seeds were infused with 100 ml of sterile water by the sudden release of 680 mm of mercury vacuum. Seeds were agitated at 150 rpm for 2 hr on an orbital shaker, and the suspensions were centrifuged at 4,000 *g* for 30 min. Pellets were resuspended in 5 ml of supernatant and the mixture was infiltrated with a syringe and needle into healthy leaves of southern pea. Bacteria recovered from lesions that developed in 5–20 days were identified as *X. campestris* pv. *vignicola*. Incidence of bacterial blight in field plots was significantly greater in cultivars that were grown from heavily infested seeds. Bacteria were isolated from seed produced in Florida, Georgia, and Texas, as well as in California.

Additional key word: cowpea

The incidence and importance of bacterial blight and canker of southern pea (*Vigna unguiculata* (L.) Walp) caused by *Xanthomonas campestris* pv. *vignicola* (Burkholder) Dye have increased in Georgia in recent years. Seedborne inocula initiate epidemics of a number of bacterial plant diseases and play an important epidemiologic role in bacterial diseases of leguminous plants (2–5). Although Shekhawat and Patel (7) reported transmission of *X. campestris* pv. *vignicola* through experimentally infested seed, the bacterium has not been demonstrated in natural seed lots.

The purpose of this report is to provide data on detection of the bacterium from naturally infested southern pea seed. A major problem in the detection of phytopathogenic bacteria in a natural habitat such as seed is competition from fast-growing, saprophytic bacteria that usually outnumber the pathogen. Although a selective medium (SX agar) exists for *X. campestris* pv. *campestris* and certain other *X. campestris* pathovars, *X. campestris* pv. *vignicola* is similar to *X. campestris* pv. *phaseoli* in that it exhibits limited growth only in mass streak on SX agar (6). Because a suitable selective medium is not available, we used a modification of the host plant bioassay method described by Kennedy (3) to detect *X. campestris* pv. *vignicola* in seed.

MATERIALS AND METHODS

Seeds of 20 cultivars of *V. unguiculata* were obtained from a commercial outlet (Dixie Seed Company, Ochlocknee, GA 31733). A portion of each seed lot was planted with a Precision Garden Seeder (Earthway Products Inc., Bristol, IN 46507) in Tifton loamy sand on 5 August 1980. Four replicates, each consisting of four 6.2-m rows of each cultivar, were arranged in a randomized complete block design. Plants were thinned to a 10-cm spacing. Plots were fertilized with 5-10-15 of N-P-K at 225 kg/ha incorporated before planting and again as a sidedressing 4 wk after planting.

Disease ratings were recorded when disease symptoms appeared 3 wk after plant emergence. Numbers of leaflets with bacterial blight in each 6.2-m row were recorded weekly until the peas were mature. After seeding, remnant seeds of all cultivars except Dixie Lee were evaluated for seedborne *X. campestris* pv. *vignicola*. Seeds were surface disinfested for 5 min with 95% ethanol, and samples of 100 seeds were randomly sampled and submerged in 100 ml of sterile distilled water in 250-ml beakers. Three replicates of seed of each cultivar were placed in a vacuum chamber (National Vacuum Oven, National Appliance Company, Portland, OR 97208) for 5 min at 680 mm of mercury vacuum. Vacuum was released suddenly (2 sec) to infuse seeds with sterile water. Samples were reexposed to 680 mm of vacuum for 5 sec, and the vacuum was

Accepted for publication 27 April 1981.

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0191-2917/82/01002003/$03.00/0

again released suddenly. Seeds were then agitated at 150 rpm on an orbital shaker for 2 hr, and the suspensions were decanted into two 50-ml centrifuge tubes and centrifuged at 4,000 *g* for 30 min. Pellets from the two tubes were combined and resuspended in 5 ml of their supernatant, and the suspension was infiltrated into leaves of California Blackeye no. 3 and Mississippi Silver plants with a syringe fitted with a 27 gauge needle. After infiltration, plants were maintained in a greenhouse at 32–38 C. Leaves were examined daily for 30 days.

Yellow-pigmented bacteria isolated onto nutrient agar from lesions typical of bacterial blight were characterized morphologically and physiologically and tested for pathogenicity (1). Sensitivity of the bioassay was tested by recovering *X. campestris* pv. *vignicola* from a seed lot of Mississippi Silver that was experimentally infested by vacuum infiltration at 680 mm of vacuum with tenfold dilutions of bacterial suspensions ranging from 10^2–10^8 colony-forming units (CFU) per milliliter. Recovery from the experimentally infested seed was compared with the initial corresponding inoculum levels of pure bacterial cultures suspended in water. The experimentally infested seed lot of Mississippi Silver, which was different from that used in the field trial, had previously been tested by bioassay and was deemed free of *X. campestris* pv. *vignicola*. Experimentally infested seeds were assayed the same day as infestation after being air-dried.

RESULTS

Typical bacterial blight symptoms developed in leaves of assay plants for 12 of 19 southern pea seed lots tested (Table 1). Symptoms appeared 5–20 days after infiltration. Yellow-pigmented bacteria isolated from the lesions were aerobic, Gram-negative rods with single polar flagella. All isolates were positive for the hydrolysis of casein, egg yolk, gelatin, and starch. They were also positive for catalase, lecithinase, and production of hydrogen sulfide. All isolates were negative for the presence of oxidase, arginine dihydrolase, and nitrate reductase. Isolates with the above characteristics induced typical bacterial blight symptoms when reinoculated onto southern pea. Responses of the isolates to these tests were identical to those of *X. campestris* pv. *vignicola* ICPB isolate XV 19. Based on these criteria, bacteria isolated from natural seed infections were identified as *X. campestris* pv. *vignicola*.

Tenfold serial dilutions of the 5-ml sample suspensions were also used as inocula to determine the lowest dilution for each positive seed lot in which *X. campestris* pv. *vignicola* could be detected (Table 1). Bacteria were detected in the different seed lots at dilutions of 10^{-3}–10^{-8}. The time required for initial symptoms to develop was generally related to the dilution end point (Table 1).

X. campestris pv. *vignicola* was detected at all concentrations tested when bacterial suspensions ranging from 10^2–10^8 CFU/ml were introduced into a pathogen-free seed lot of Mississippi Silver. Serial dilutions of bacteria recovered from seed produced varying degrees of symptoms related to the initial concentration of inoculum. Reactions from the seed recovery inoculum were more intense than a corresponding inoculum level of bacterial suspension infiltrated directly into leaves.

Although the extreme hot and dry conditions of the summer of 1980 were not generally favorable for bacterial blight development, substantial disease occurred in the field plots at Tifton. Disease incidence was higher in Colossus, Texas Cream 40, and Mixed Iron and Clay than in the other cultivars (Table 1). These cultivars not only had a substantially higher number of leaflets with bacterial blight, but the initial distribution of disease was widespread in the four replicates. Disease in the other cultivars was concentrated in scattered foci and rarely occurred in more than two and never in all four replicates.

DISCUSSION

This method appears to be very sensitive for detection of *X. campestris* pv. *vignicola* in southern pea seed. The procedure allows for a 20-fold concentration of the seed wash water, which explains why reactions were more intense with the seed wash of experimentally infested seed than with the initial suspension of bacteria of corresponding inoculum level. The ability to recover known quantities of bacteria from experimentally infested seed may or may not relate to the numbers of bacteria recovered from natural infections, because bacteria vacuumed in from a suspension could be in a different physiologic state than those in a natural infection. However, known levels of bacteria at low concentrations were detected with this method without interference from natural seed microflora, which is the major problem with using standard microbiologic media to recover bacteria from natural habitats.

The effect of concentration during centrifugation should also be considered when determining the dilution end point of inoculum. One-hundred seeds of Colossus or Texas Cream 40 with a dilution end point of 10^{-8} would have a minimum inoculum load of 5×10^6 CFU/ml. Cultivars such as White Acre and California Blackeye no. 5 would have minimum inoculum levels of 5×10^2 CFU/ml and 0.5×10^2 CFU/ml, respectively. Our results are similar to Kennedy's (3) findings in that symptoms developed faster with higher levels of inoculum.

It is of interest that symptoms did not appear in field plots until 3 wk after emergence. A similar phenomenon has been observed with the cultivar Coronet (R. D. Gitaitis, *unpublished data*), which is known to contain seedborne *X. campestris* pv. *vignicola*, and with Texas

Table 1. Bioassay for the detection of *Xanthomonas campestris* pv. *vignicola* in seed lots and incidence of bacterial blight on southern peas in Georgia

Cultivar	Seed origin	Bioassay: Days for symptom development[a]	Bioassay: Dilution end point[b]	Disease incidence in the field[c]
Colossus	Texas	5	10^{-8}	69.25 x[d]
Texas Cream 40	Texas	5	10^{-8}	40.25 y
Mixed Iron and Clay	Florida	5	10^{-7}	27.75 y
SA Dandy	Georgia	5	10^{-7}	1.75 z
Purplehull 49	Texas	8	10^{-4}	12.25 z
Zippercream	Texas	8	10^{-5}	0.00 z
Knuckle Purplehull	Texas	9	ND	2.25 z
White Acre	Texas	10	10^{-4}	4.00 z
Brown Crowder	Texas	10	10^{-5}	1.50 z
Mississippi Silver	Georgia	15	ND	2.25 z
Speckled Purplehull	Texas	15	ND	6.00 z
California Blackeye no. 5	California	20	10^{-3}	6.50 z
California Blackeye no. 3	California	···	NT	1.00 z
Texas Cream 12	Texas	···	NT	6.00 z
Tennessee White Crowder	Mexico	···	NT	2.00 z
Big Boy	Texas	···	NT	1.00 z
Calico	Texas	···	NT	0.00 z
Pinkeye Purplehull	Georgia	···	NT	0.50 z
Bluegoose	Georgia	···	NT	2.00 z
Dixie Lee	Georgia	ND	NT	0.75 z

[a] ··· = No symptoms after 30-day incubation period, three replicates.
[b] ND = No data because of insufficient seed supply; NT = not tested because bacteria were not detected in the concentrated seed wash.
[c] Each value is a mean of four replicates expressed as the number of leaflets with bacterial blight in a 6.2-m row 3 wk after emergence.
[d] Mean separation in columns by Duncan's multiple range test at the 5% probability level.

Cream 40 planted in the greenhouse. Control strategies for bacterial plant diseases often include the concept of reducing initial inoculum, which can have the effect of delaying the onset of an epidemic (8). Considering the development of southern peas for 3 wk before the appearance of symptoms, the lower the level that seedborne inoculum becomes, the greater the chances are of escaping severe damage.

During the course of this study, the disease spread only during a 2-wk period; however, under other environmental conditions, bacterial blight of southern pea has caused severe losses. Whether the 3-wk delay in onset of symptoms was an artifact of the summer of 1980 or not, the close association between the degree of seed infestation and disease severity and distribution in the field indicates that the use of pathogen-free seed would be of value in the culture of southern peas in Georgia.

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