Colonization of Cotton Buds by *Xanthomonas campestris* pv. *malvacearum*

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ABSTRACT

Wrather, J. A., Sappenfield, W. P., and Baldwin, C. H. 1986. Colonization of cotton buds by *Xanthomonas campestris* pv. *malvacearum*. Plant Disease 70:551-552.

Xanthomonas campestris pv. *malvacearum* (*X. c. malvacearum*) was found within the buds of field-grown cotton in two consecutive years. In greenhouse studies, *X. c. malvacearum* race 12 survived within the terminal buds of susceptible Auburn M cotton for at least 10 days but did not survive within the buds of resistant cotton line 101-102B. Auburn M seed inoculated or naturally infested with *X. c. malvacearum* produced seedlings with the pathovar within the terminal buds. Simulated rain spread *X. c. malvacearum* from these buds to the plant stem.

Bacterial blight of cotton caused by *Xanthomonas campestris* pv. *malvacearum* (*X. c. malvacearum*) (Smith) Dye can be a devastating disease in the upper midsouthern region of the United States. The bacterium survives from crop to crop in infested cotton residue (2) and seed (3). Wind, rain, dew, and irrigation water are the primary vectors for *X. c. malvacearum*. The epidemiology of *Pseudomonas syringae* pv. *glycinea* (*P. s. glycinea*) is very similar to that of *X. c. malvacearum*. Leben et al (8) have demonstrated that *P. s. glycinea* can multiply within symptomless buds of susceptible soybean and have suggested that this resident phase of *P. s. glycinea* may have a role in the epidemiology of soybean bacterial blight. Our purpose was to determine if *X. c. malvacearum* could survive within buds of susceptible and resistant cotton and if it could be found within field-grown cotton buds.

MATERIALS AND METHODS

A culture of *X. c. malvacearum* race 12 obtained from L. S. Bird (Texas A&M University, College Station) was used in all experiments. The culture was lyophilized and separate portions were stored at −4 C. Before each experiment, a portion of the lyophilized bacteria was suspended in sterile distilled water, streaked onto the surface of nutrient agar, and incubated for 3 days at 27 C. A sample of this culture was streaked onto the surface of fresh nutrient agar and incubated as before. The resulting bacterial growth was scraped from the agar surface and suspended in sterile distilled water. The suspension was centrifuged at 12,000 *g* for 20 min. The pellet was resuspended in sterile distilled water and the bacteria concentration adjusted to 10^6 cells per milliliter.

Isolation of *X. c. malvacearum* from terminal buds of field-grown cotton. The terminal buds from 200 cotton plants (cultivar DPL 55) were collected from southeastern Missouri fields in July of two consecutive years. The bud was considered to be a small protuberance at the undeveloped shoot terminal consisting of the apical meristem, leaf primordia, and young leaves shorter than 2 mm. Each bud was ground separately in 10 ml of sterile water with a mortar and pestle. Three 0.1-ml aliquots of the homogenate were spread over the surface of nutrient agar in separate petri plates. The plates were incubated as described previously, and all *X. c. malvacearum* colonies were counted and tested for pathogenicity (1). Pathogenicity was tested by inoculating Auburn M cotyledons with a water suspension of the bacteria. The inoculation was made by wounding the cotyledons' lower surface with a bacteria-soaked cotton swab. The *X. c. malvacearum*-inoculated cotyledons became water-soaked 7 days later.

Population dynamics of *X. c. malvacearum* within cotton buds. Acid-delinted seed of *Gossypium hirsutum* L. cv. Auburn M and line 101-102B, susceptible and resistant to *X. c. malvacearum* race 12, respectively, were planted in peat pellets and maintained in the greenhouse at 26 ± 3 C and 80–90% RH. The buds of 180 10-day-old Auburn M and line 101-102B seedlings were inoculated with 0.1 ml of a suspension of *X. c. malvacearum* (10^6 cells per milliliter). Sterile water was similarly placed onto buds of control plants. Plants were maintained in the greenhouse in a completely randomized design, and water was added to the peat pellets daily. Overhead watering was not done. The population of bacteria within the buds was assayed 1, 3, 5, 7, and 10 days after inoculation. At each sampling, 30 buds from each treatment were collected and processed separately as described previously. The number of bacteria per bud was thus determined. The experiment was conducted twice, and the data from the two experiments were pooled for analysis. The data were analyzed statistically and LSD was used to compare means. The buds of 30 plants from each treatment were not sampled. Those plants were maintained in the greenhouse for 6 wk postinoculation and observed periodically for symptoms of bacterial blight.

Detection of *X. c. malvacearum* within buds of seedlings from inoculated seed. Acid-delinted Auburn M seed previously soaked for 5 min in a suspension of *X. c. malvacearum* race 12 (10^6 cells per milliliter) or in sterile distilled water and Auburn M seed naturally infested with *X. c. malvacearum* (25% of the seed harbored *X. c. malvacearum*) were planted in peat pellets and maintained in the greenhouse. Fifty terminal buds of seedlings from each of the three treatments were assayed 10 days after planting for *X. c. malvacearum* as described. The experiment was conducted twice, and the percentage of seedling buds with *X. c. malvacearum* was calculated.

Spread of *X. c. malvacearum* from cotton buds by simulated rain. Buds of 100 14-day-old Auburn M seedlings were inoculated with *X. c. malvacearum* or sterile water as described and maintained in the greenhouse. Seven days later, drops

Published as Journal Series Paper 8692 of the University of Missouri Agricultural Experiment Station.

Accepted for publication 19 November 1985.

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Table 1. Population dynamics (bacteria per bud) of *Xanthomonas campestris* pv. *malvacearum* race 12 within terminal buds of cotton cultivar Auburn M and line 101-102B[z]

Cotton line	Days after inoculation				
	1	3	5	7	10
Auburn M	2,000 a	10,000 a	11,000 a	11,000 a	10,000 a
101-102B	2,100 a	2,200 b	1,000 b	500 b	0 b

[z] Cultivar Auburn M is susceptible to *X. c. malvacearum* race 12 and line 101-102B is resistant. Means within columns are not significantly different if followed by the same letter (LSD = 1,800 at $P = 0.05$).

of sterile tap water, simulating rain, were applied to the buds or stems just below the bud until runoff. The runoff water was collected from the stems, spread over the surface of nutrient agar, and incubated. The *X. c. malvacearum* colonies were tested for pathogenicity as described. The experiment was conducted twice, and the percentage of runoff samples containing *X. c. malvacearum* was calculated.

RESULTS

Race 12 of *X. c. malvacearum* survived for at least 10 days within Auburn M buds but did not survive within 101-102B buds (Table 1). The number of *X. c. malvacearum* cells within 101-102B buds was significantly less than within Auburn M buds 3, 5, 7, and 10 days after inoculation. The *X. c. malvacearum* population within Auburn M buds increased for 3 days after inoculation and remained constant for the next 7 days. The bacteria population within 101-102B buds declined until no bacteria were detectable 10 days after inoculation. No bacteria other than *X. c. malvacearum* were isolated from the buds, and *X. c. malvacearum* was not isolated from uninoculated buds. No symptoms of bacterial blight were observed on the buds when the experiments were terminated 6 wk after inoculation. However, leaf lesions were observed on some plants 3 wk after inoculation. None of the water-inoculated plants had disease symptoms.

Simulated rain did wash *X. c. malvacearum* from some Auburn M buds. We found *X. c. malvacearum* in 70 of the 200 runoff samples collected below inoculated buds. We did not find *X. c. malvacearum* in runoff samples collected below uninoculated buds or in samples of water applied to stems only.

We assayed 200 buds of field-grown DPL 55 cotton for *X. c. malvacearum* in July of two consecutive years and found *X. c. malvacearum* within 4 and 10% of the buds the first and second year, respectively. None of the buds had symptoms of bacterial blight; however, some plants had foliar symptoms. Very few other bacteria, and no fungi, were isolated from these buds. All *X. c. malvacearum* isolates were pathogenic.

A source of *X. c. malvacearum* within buds in nature may be infested seed. We conducted experiments with cotton seed naturally or artificially infested with *X. c. malvacearum* and found that the developing seedlings had *X. c. malvacearum* within their buds (four and six buds per 100 tested, respectively). Buds of seedlings from bacteria-free seed did not harbor *X. c. malvacearum*. None of the buds showed symptoms of bacterial blight.

DISCUSSION

In two consecutive years, *X. c. malvacearum* was found within symptomless terminal buds of field-grown cotton. That discovery led to greenhouse studies to determine if *X. c. malvacearum* could survive within cotton buds and if it could be spread from the buds by water. These studies demonstrate that in a greenhouse situation, *X. c. malvacearum* can survive within buds of susceptible yet symptomless Auburn M seedlings. The population of *X. c. malvacearum* did increase slightly within the susceptible cotton buds and is probably in a resident phase (7). The inability of *X. c. malvacearum* race 12 to survive within 101-102B buds is probably due to lack of food or an otherwise inhospitable environment. The reason may be inherent.

The location of *X. c. malvacearum* within the buds is unknown. They may be living as epiphytes on the tissue surface (5,6) or inside the tissue. If *X. c. malvacearum* is surviving inside the Auburn M bud, it is doing so without causing symptoms. We were able to wash some *X. c. malvacearum* from the buds by shaking in water for 1 hr, but the number of bacteria detected per bud was lower than when the buds were ground (*unpublished*). Burr (4) observed that grinding apple buds was a more reliable assay for *P. s. populans* than washing. Washing buds is evidently an unreliable technique for estimating the number of bacteria per bud. Neither technique, grinding or washing, is adequate to determine the source of bacteria.

The manner by which *X. c. malvacearum* from infested seed reached the seedling buds is unknown. The bacteria may have become established within the bud at the time of seed germination. Precautions were taken in our experiments to prevent movement of bacteria to the bud by insects and splashing water. It is evident that *X. c. malvacearum* within the buds can come from the seed. The *X. c. malvacearum* found in symptomless buds of susceptible field-grown DPL cotton may have come from infested seed or been vectored to the bud from some source by rain or insects.

The *X. c. malvacearum* surviving in cotton buds of greenhouse-grown plants could serve as inoculum for other nearby plants. The bacterium was moved readily from within buds to the stem by water and might spread to other plants during watering. Further investigation may reveal that the survival of *X. c. malvacearum* within buds of susceptible field-grown cotton is important in the epidemiology of cotton bacterial blight.

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