Altered Host Specificity in Race 1 of Xanthomonas malvacearum by Passage Through a Resistant Variety of Gossypium hirsutum

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ABSTRACT

A single-cell culture of race 1 of *Xanthomonas malvacearum* was inoculated to young leaves of Stoneville 20, a resistant variety of *Gossypium hirsutum*. Isolations were made from small, partially or wholly necrotic lesions 14 and 56 days later. No detectable change in colony traits or in pathogenicity occurred in race 1 in 14 days (even after three successive passes through leaves), but colonies similar to race 2 were apparent in isolations made after 56 days (on the first pass). Purification of buff (race 1 type) and yellow (race 2 type) colonies and inoculation to Stoneville 20 demonstrated that the yellow colonies were race 2. Race 2 comprised 77% of the cells in necrotic lesions 56 days after inoculation. Since no spontaneous change from race 1 to race 2 had been observed in culture over a 10-year period, and since none was apparent in hundreds of isolations from lesions from susceptible varieties, the altered specificity is associated with the B_7 (resistance) gene in Stoneville 20. Phytopathology 60: 258-260.

The host specificity of xanthomonads was reported by Dye (4) to change at the species level after successive inoculation and recovery from bean plants. Logan (6) and Schnathorst (8) could not confirm Dye's results, in that they were unable to obtain xanthomonads that would cause progressive disease in bean after repeated inoculation and isolation. Some xanthomonads cause the early symptoms also induced by *Xanthomonas phaseoli* (E. F. Sm.) Dows. in bean (*Phaseolus vulgaris* L. 'Bountiful'), but fail to progress after 7 days, and small hypersensitive lesions result (8). In addition, when inoculated to bean plants, xanthomonads other than *X. phaseoli* induce lesions only if the leaf tissue is water-soaked (8). On their own respective hosts, the usual pathogens do not require water-soaked tissue.

Failure to induce a change in host specificity by repeated passage through bean leaves might be due to the method employed. Of primary concern was the possibility that the bacteria and plant cells had not been in contact with each other long enough for a change to occur.

This possibility was tested in additional experiments conducted with bean callus tissue. Tissue cultures of bean (Bountiful) were grown on modified White's medium in 250-ml Erlenmeyer flasks. After 3 weeks of growth, the tissues were moderately fragmented with a sterile glass rod to expose cells, and inoculated with suspensions of the following species: *X. malvacearum* (E. F. Sm.) Dows. races 1 and 2; *X. pruni* (E. F. Sm.) Dows.; and *X. transluscens* f. sp. *cerealis* Hagb. After a 3-week incubation with the tissue, each bacterial species was reisolated and inoculated by methods previously described (8) to trifoliolate leaves of Bountiful bean plants. This procedure was repeated four times with the original culture. There was no indication that any of the above species had changed in specificity and would attack bean plants.

These results, coupled with earlier ones (8), suggest that changes in host specificity at the species level might be too drastic to expect with present methodology. Consequently, attempts were made to alter host specificity at the race level by passing race 1 of *X. malvacearum* through leaves of Stoneville 20, a cotton variety with resistance to race 1 conditioned primarily by the B_7 gene (5). This paper reports successful alteration of the host specificity of race 1 to that exhibited by race 2, and the methods required to accomplish the change.

Methods and Results.—A culture of race 1 of *X. malvacearum* from cotton (*Gossypium hirsutum* L.) was single-celled by methods of DeVay & Schnathorst (2), and its reaction was verified on three race-differentiating cotton varieties (12). A 5-day-old culture grown on carrot agar (8) slants was flooded with 10 ml of sterile glass-distilled water, and the cells were placed in suspension by agitating the culture tube with a vortex mixer. Suspensions prepared in this manner generally contained 1.5×10^8 viable cells/ml. The suspension was delivered to the undersurface of leaves of the resistant Stoneville 20 variety with a sterile 1-ml pipette at midday and spread evenly over the entire surface of the leaf. Young (7 to 9 days old) true leaves of five plants were inoculated. The plants were covered for 4 days with a polyethylene bag held in place by a rubber band placed on the rim of 4-inch pots, and were held at 26 C on the greenhouse bench. Several plants of the highly susceptible Acala 4-42 variety were inoculated similarly to verify the virulence of the inoculum. Subsequent inoculations were made in the same manner. No other race of *X. malvacearum* or species of *Xanthomonas* was present in the greenhouse or nearby greenhouses during these experiments.

For isolation of bacteria from leaf tissue, one to several lesions were crushed with a sterile glass rod in 0.25 ml of sterile glass-distilled water in a sterile test tube. Thirty minutes or more were allowed for diffusion of bacteria from the crushed tissue. A bacterial loop was used to streak the suspension over the surface of carrot agar, or the suspension was serially diluted to 10^{-6} and plated on carrot agar.

In initial experiments, isolations were made from small (0.5 mm), partially necrotic lesions 14 days after inoculation. Race 1 was isolated and reinoculated three times to Stoneville 20. Since my culture of race 2 has a characteristic colony morphology and is more deeply pigmented than any of the race 1 isolates in my collec-

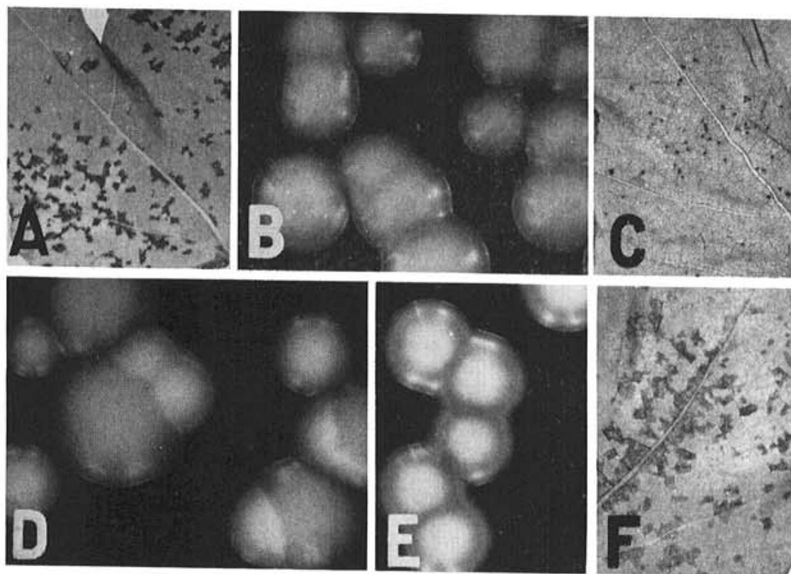

Fig. 1. Illustration of the steps involved in altering host specificity of race 1 of *Xanthomonas malvacearum*. Lesions caused by race 1 on highly susceptible Acala 4-42 **A)** yield typical race 1 colonies as in **B)**. When race 1 is inoculated to resistant Stoneville 20, however, small hypersensitive lesions are formed, as in **C)**. Upon isolation from Stoneville 20, 8 weeks after inoculation, a mixed population of bacteria is apparent, as in **D)**, with deeply pigmented colonies appearing similar to a known culture of race 2 **E)**. After purification and inoculation of the two colony types to a differential varietal series, the race-2 colony type proved pathogenic on Stoneville 20, as in **F)**, indicating that it is identical to a known culture of race 2.

tion (Fig. 1-E), colonies with race-2 traits were sought on streaked plates and dilution plates. There was no indication of a change in colony type or pigmentation or increase in virulence after the third passage through Stoneville 20.

Instead of making isolations 14 days after inoculation, lesions were allowed to become necrotic and dry (Fig. 1-C). Leaves were harvested 8 weeks after inoculation. They were dried between paper towels for at least a week, and isolations made as before.

Colony counts on dilution plates indicated that the small dry necrotic lesions caused by race 1 on Stoneville 20 contained $1\text{-}3 \times 10^6$ viable bacteria. The numbers of cells per lesion in this case averaged one-third to one-half those found in single dried lesions of comparable age from a highly susceptible variety (9). After 7 days of incubation, a colony type different from that typical of race 1 was apparent on dilution plates and on streaked plates (Fig. 1-D). The variant appeared similar to colonies of race 2 (Fig. 1-E). In hundreds of isolations of *X. malvacearum* from leaves of susceptible varieties (7, 9, 10, 12), no variants had been observed. Similarly, no variants of this type had been observed in cultures of race 1 on carrot agar over a 10-year period. The buff (race 1 type) and deep-yellow (race 2) type colonies were purified by dilution and selection. Inoculation of each type to the cotton differentials Stoneville 20, Texas S9, and Mebane B1 demonstrated that the buff colonies were race 1 and the deep-yellow ones were race 2 of *X. malvacearum* (Fig. 1-B, E). Subsequent isolations from similar single lesions indicated that both races were present in the majority of lesions on Stoneville 20 leaves. Additional inoculations and isolations by similar methods showed that race 2 was commonly present in 8-week-old lesions after inoculation of Stoneville 20 with race 1.

The percentage of yellow race-2 colonies in the mixture of races 1 and 2 isolated from the 8-week-old single lesions was determined by counting the total number of each colony type on dilution plates. Race 2 comprised 77% of the mixture (Fig. 1-D), although only race 1 had been inoculated originally.

Discussion.—The results indicate that *X. malvacearum* can change host specificity at the race level relatively rapidly and frequently. Of possible importance in the change may be the single major gene (B_7) for resistance present in Stoneville 20. Future studies will be designed to determine whether a similar change occurs when races 1 and 2 are inoculated to Mebane B1 which is endowed with major genes B_2 and B_3 and is resistant to both races (5). Two-factor resistance may retard or prevent changes in host specificity.

Adequate quantitative data are lacking to determine whether the change involves a mutation or transformation by the host. Studies are currently in progress to obtain data on the frequency of the change from race 1 to race 2, and whether such a change can be induced by certain host constituents. Transformation of virulence by the host is of interest in this regard because of the possibility of uptake and incorporation of host antigens by *X. malvacearum*, some of which appear to be shared with *G. hirsutum* (3, 11).

Of importance to plant breeders is the possibility that the resistance of varieties to angular leaf spot may last for very short periods if only one gene is involved. In spite of the fact that lesions caused by race 1 are

quite small and become necrotic sooner on Stoneville 20 than on susceptible varieties, the pathogen is present in fairly high populations, and could overwinter on cotton debris. Varieties containing the B_7 gene could feasibly succumb to an attack by race 2 in the second season.

It is apparent that a major factor involved in the change in race reaction in *X. malvacearum* is the length of time that the relatively innocuous race is in contact with resistant plant tissue. Two weeks were insufficient for a detectable change in this study, even with three successive passes through resistant leaf tissue, whereas 8 weeks resulted in the formation of a high population of a new race on the first pass. A similar approach could be used in attempts to alter the specificity of xanthomonads at the species level. However, in earlier studies (8), nonpathogenic xanthomonads were difficult to recover from bean tissue 14 days after inoculation even when leaf tissue was water-soaked prior to inoculation. Any xanthomonads altered by passage through bean with the above approach would doubtless be recovered in very low numbers if at all.

Although I reported (8) that altered specificity was undetected in seven passes of several xanthomonads through *Phaseolus vulgaris,* it is now apparent that specificity can be changed at the race level by one pass of an innocuous race through a resistant variety of *G. hirsutum.*

These results agree with the suggestion in 1963 by Brinkerhoff (1) that development of new races of *X. malvacearum* may be related to host reaction, and with Brinkerhoff's (*personal communication*) more recent work on the development of new pathotypes in resistant cotton varieties.

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