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Development of Immunity to Bacterial Blight of

The development of stable resistance to disease is a matter of serious concern in plant breeding. Generally, polygenes are thought to confer stable resistance, and qualitative genes to confer nonstable resistance (22). As Robinson (23) has emphasized, however, exceptions exist in which single-gene resistance has remained stable for considerable periods of time. Nelson (22) presented a comprehensive review of concepts concerning plant disease resistance.

For over 20 years, immunity to bacterial blight, incited by *Xanthomonas campestris* pv. *malvacearum* (Smith) Dye (formerly *X. malvacearum* (E. F. Sm.) Dows.) (Fig. 1), has been stable in breeding lines of upland cotton (*Gossypium hirsutum* L.). During that period, immune lines have been tested across the U.S. Cotton Belt and in South Africa, Rhodesia, Uganda, Sudan, India, and Pakistan (6,18). In none of those locations until recently, and then *only* in the Upper Volta and the Sudan (10), has there been any indication from either natural infestations or artificial inoculations that this immunity was vulnerable to different races or to new mutations of *X. campestris* pv. *malvacearum* (hereafter referred to as *X. malvacearum*). In contrast to immunity (as conditioned by multiple genes), resistance to bacterial blight of cotton (as conferred by single genes) has repeatedly proved to be ineffective because of the appearance of new races of the pathogen (6). Our approach to understanding the nature of blight immunity has been multidisciplinary. In recent years, we have published findings on the pathological, biochemical, anatomical, and genetic factors responsible for this immunity (eg, 1,8,11,13,14).

Approved for publication by the Langston Agricultural Research Programs as Journal Series J101 and by the Oklahoma Agricultural Experiment Station as Journal Article No. 4409. Supported by USDA Cooperative State Research Service grants 616-15-41, 8085-15-7, and 8287-15-12 and by the Oklahoma Agricultural Experiment Station.

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Definitions of Terms

We define "immunity" to bacterial blight of cotton as a response to all 18 known races (individually or combined)

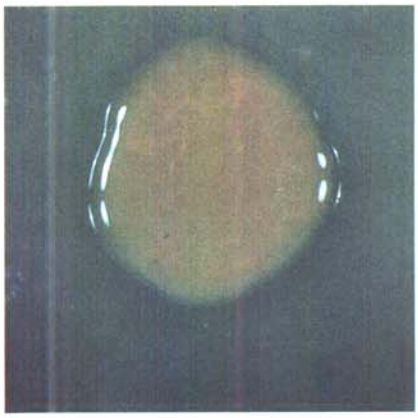

Fig. 1. Single colony of *Xanthomonas campestris* pv. *malvacearum*, causal agent of bacterial blight of cotton.

of *X. malvacearum*, which shows no macroscopic symptoms under field levels of inoculum (Fig. 2, top row). Necrotic cells or cell clusters are visible microscopically in inoculated immune cotton leaves (Fig. 3), however, and these cell clusters occur in proportion to the inoculum level (13). Macroscopic symptoms are produced as the typical hypersensitive reaction (HR) with inoculum levels of $1 \times 10^{6-7}$ colony-forming units (cfu) per milliliter or higher (16). This level of inoculum is far in excess of typical field levels, ie, 0.5×10^{6} or lower—usually lower. Heterologous phytopathogens on cotton, including *X. campestris* pv. *campestris*, pv. *vesicatoria*, pv. *oryzae*, and pv. *phaseoli* and *Pseudomonas syringae* pv. *pisi*, incite the HR in both fully bacterial blight-immune and blight-susceptible cotton at comparable inoculum levels of $1 \times 10^{6-7}$ cfu/ml or higher. Also, these bacteria induce necrotic cells at inoculum levels below that for the macroscopic HR in both blight-immune and blight-susceptible cotton. Thus, the reactions to heterologous

Fig. 2. Blight-immune line Im 216 (top row; left leaf sustained some physical damage) and blight-susceptible line Ac 44 (bottom row). Leaves inoculated with race 1 (left column), race 2 (middle column), and a mixture of race 1 and race 2 (right column).

Cotton and Its Implications for Other Diseases

phytopathogens in susceptible and immune cottons are similar in gross appearance to those of *X. malvacearum* in blight-immune cotton. In immune cotton, *X. malvacearum* has essentially been demoted to the status of a nonpathogen!

We use the term "resistance" to indicate a macroscopically visible response characterized by small, dry, round-to-angular lesions on the leaves (Fig. 4 A–D). These macroscopic lesions are induced in blight-resistant cotton by *X. malvacearum* at field levels of inoculum, but not by heterologous phytopathogens. Necrotic cells, which develop before the macroscopic symptoms appear, are also induced by *X. malvacearum* in resistant cotton at field levels of inoculum, and the macroscopically visible HR is induced at higher inoculum levels as well. The necrotic responses are comparable to those induced in blight-immune cotton, except that they develop more slowly. At the higher inoculum levels, heterologous phytopathogens also incite necrotic cells and the macroscopically visible HR in blight-resistant cotton.

We define "susceptibility" to bacterial blight as a macroscopic response characterized by relatively large (4–6 mm in diameter), angular, water-soaked lesions that develop on the leaves 2–3 weeks after inoculation (Fig. 4F). The pathogen also

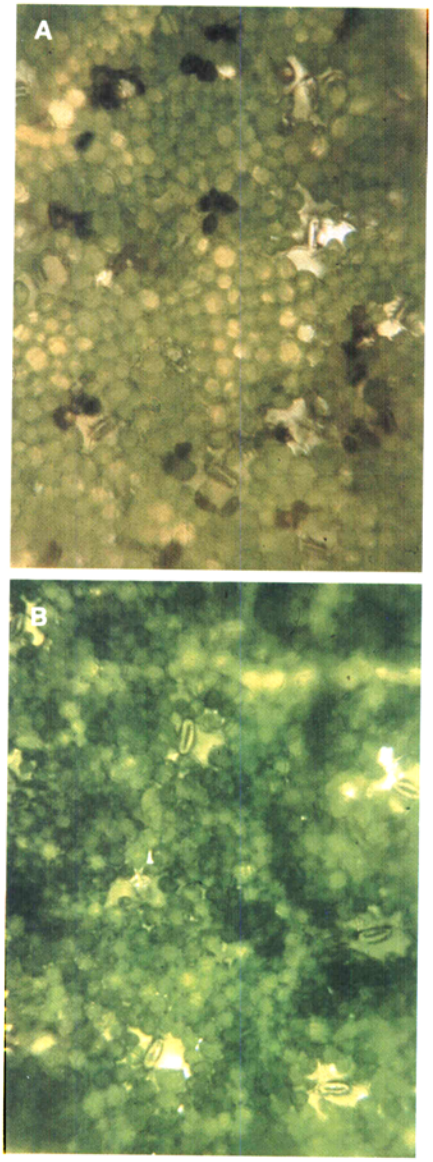

Fig. 3. Microscopic view of infiltrated leaves of the blight-immune line Im 216: (A) Necrosis in leaf inoculated with *Xanthomonas campestris* pv. *malvacearum* and (B) noninoculated control.

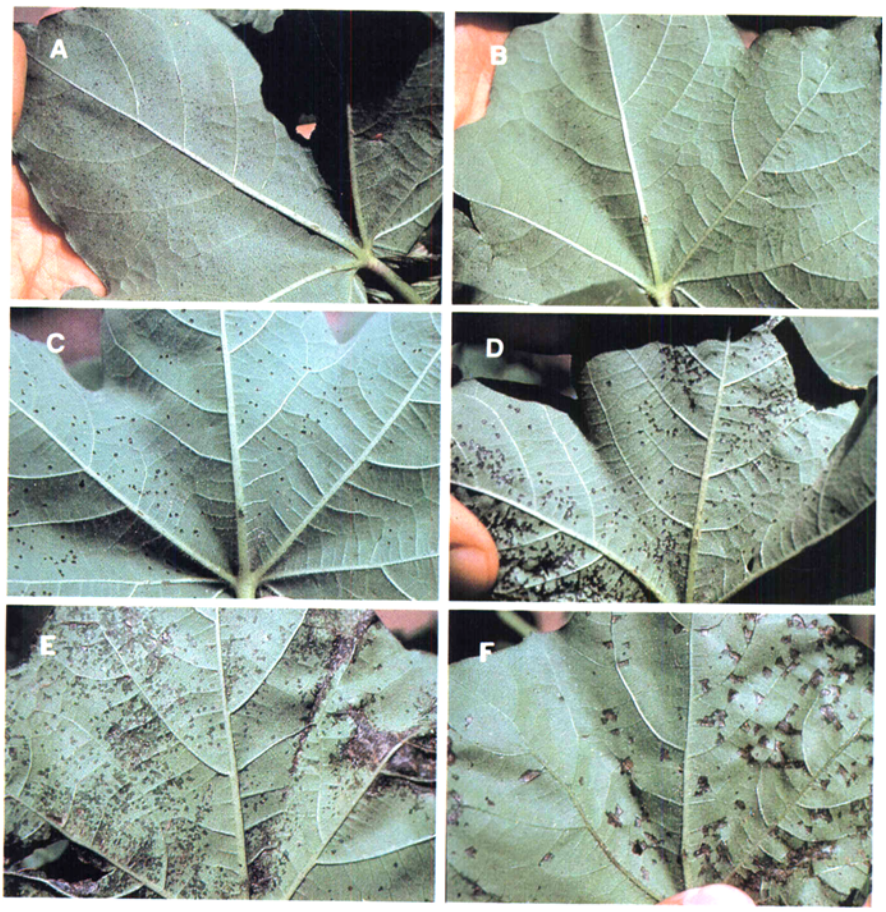

Fig. 4. Scoring system used in Oklahoma for bacterial blight in leaves: (A) 0.1, (B) 0.2, (C) 1.0, (D) 1.2, (E) 2.3, and (F) 4.0; see top row in Figure 2 for immune score (0.0). Reactions shown are resistant (A–D), moderately susceptible (E), and fully susceptible (F).

infects all other aboveground portions of the plant, including bolls (Fig. 5) and stems (Fig. 6).

How Immunity Was Developed

L. S. Bird (3,4) first developed immunity at Texas A&M University in about 1960 in 101-102B, a line of *G. hirsutum*. This line was developed by crossing Empire WR (*G. hirsutum*) and Bar 4/16, a Sudanese line of *G. barbadense* L. containing Knight's B_2B_3 blight-resistance genes. Five backcrosses to Empire WR (which has an intermediate level of polygenic blight resistance [5]) were followed by a cross to the upland line, MVW, which may contain b_7. After each backcross, the segregating populations were screened with a mixture of races of *X. malvacearum*, and the most resistant plants were then backcrossed to the recurrent parent. Immune plants were not detected until after several backcrosses (3,6).

The dominant blight-resistance gene B_2 was originally transferred from Uganda B 31, a *G. hirsutum* line acclimatized to that country (20). The B_2 gene has since been identified in a number of upland cottons from different parts of the world (6). Knight also transferred the dominant resistance gene B_3 from *G. hirsutum* var. *punctatum* (19). When transferred into Ac 44, a fully blight-susceptible line of upland cotton, the single gene B_2 conferred an intermediate level of blight resistance to race 1. B_3 in the same genetic background conferred a relatively high level of resistance to race 1, but the B_2 resistance appears less subject to attack by mutants (8). Empire WR, the recurrent parent, has a low level of resistance that is conferred by the dominant polygenic complex, B_{Sm}, originally described in Stoneville 20 (5).

Im 216, a blight-immune line (Fig. 2, top row) developed by L. A. Brinkerhoff in Oklahoma, was selected as an immune plant from a segregating population of Bird's B_2B_3 Empire, one of the parental populations of 101-102B. Immune plants were identified by screening with a mixture of virulent races of *X. malvacearum*. Homozygous immunity to bacterial blight was obtained by inbreeding among those selections. Originally, the immune selections segregated for predominantly immune (but with a few highly resistant) plants. After several generations of selection, however, homozygous immune lines were obtained. The inocula were composed of races 1, 2, 4, and 10, which are capable (either individually as a set or combined as a mixture) of overcoming all known single genes for blight resistance (6).

Consistent and severe pathogen pressure on the segregating populations of cotton to permit the elimination of less desirable plants is crucial for the method's success. This contrasts with the

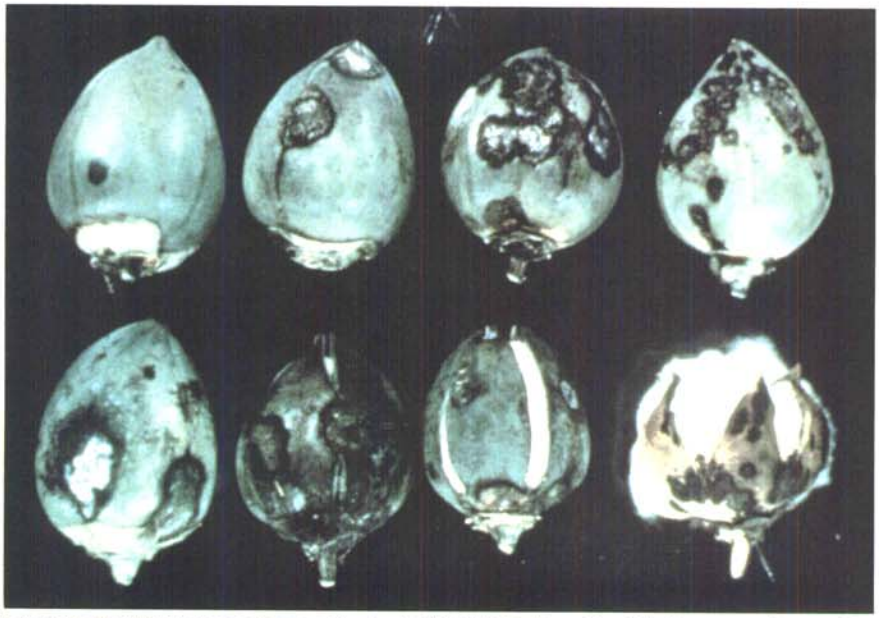

Fig. 5. Bolls with various stages of bacterial blight infection. Such bolls are predisposed to secondary invasion by other destructive microorganisms, leading to boll rots.

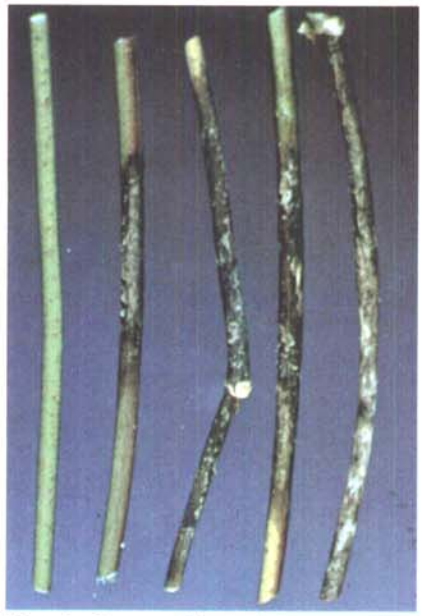

Fig. 6. Stems with various stages of bacterial blight infection; stem on left is from an uninfected plant. (Courtesy Cotton Disease Council)

Fig. 7. Transferring genes for resistance through crossing.

Fig. 8. Selfing to eliminate cross-pollination is crucial to develop and maintain immunity. Cotton sacks are applied before anthesis.

Fig. 9. *Xanthomonas campestris* pv. *malvacearum* inoculum in aqueous suspension applied under pressure to individual plants in the field.

use of mixtures of isolates of unknown identity and severity collected and used each year by Arnold et al (2) for the same disease/crop complex. Although they were undeniably successful in breeding for resistance to bacterial blight of cotton, their choice of inoculum likely prevented the attainment of immunity. It certainly could account for some of the variation in results they reported.

Inheritance of Immunity

Immunity in Im 216 is inherited as a completely dominant trait (7). The immunity has been readily transferred many times to completely susceptible genetic backgrounds by crossing (Fig. 7) and forced inbreeding (Fig. 8) of immune plants that were identified through field inoculation (Fig. 9) with a mixture of races 1, 2, 4, and 10 (and later of races 1, 2, 4, and 18) of the pathogen and scored using the system illustrated in Figures 2 and 4. The F_1, F_3, and F_5 generations are usually grown in a winter nursery in Mexico without screening. Materials are harvested there as individual selfed plants. Seed for the F_2, F_4, and F_6 generations are divided and planted in two or more nurseries in Oklahoma, where they are evaluated and selfed again (9). Homozygous immunity is usually obtained by the F_6 generation, but with large populations, immune selections from the F_2 generation occasionally prove to be homozygous. Immune plants from the F_2 and F_4 populations usually segregate, however. A situation such as this implies that a number of genes, rather than only one or two, influence the trait.

The F_2 data of a cross between immune (Im 216) and fully susceptible (Ac 44) parents fit a ratio for two dominant independently inherited genes. They also fit a ratio for two dominant and one recessive independently inherited genes (7). The latter hypothesis is considered a more likely explanation because of the observed segregation of resistance from plants previously scored as fully susceptible and because of the isolation of two homozygous lines—*instead of the one expected*—with different levels of resistance (Fig. 4D,E) from the intermediate class of a 12:3:1 ratio. Backcrosses to the susceptible parent clearly indicated two genes but could not distinguish three. Homozygous resistant and intermediate lines have been obtained from the cross of immune with susceptible, but inheritance studies where those lines were crossed to the susceptible parent or to Knight's B_2 and B_3 lines indicate that their resistance was conditioned by more than single genes. Inoculations with individual races and race mixtures also indicate more than single genes are involved. Individual blight-resistance genes have not yet been

identified in lines derived from crosses of immune and susceptible parents. Our working hypothesis is that the qualitative genes involved in Im 216 are B_2, B_3, and b_7.

Innes et al (18) conducted a diallel cross study using Bird's immune 101-102B as one of the parents and suggested that in the presence of B_2B_3, the B_{Sm} complex acted as a strong modifier and that a stable "super-gene" (15) had been synthesized in the line. Knight (19) had previously demonstrated that the B_2 and B_3 genes were located on the same chromosome with a recombination value of 32.4% in some material and that recombination was increased to near 50% in yet other lines. A chromosome inversion could drastically reduce recombination between the two loci. In fact, this may have happened in 101-102B and would explain many of the genetic segregations observed (L. S. Bird, *unpublished*). El-Zik and Bird (12) found that the B_{Sm} polygenic complex greatly enhanced resistance when it was combined with the blight-resistance gene B_4 or with the gene combinations of B_2B_3, B_2B_6, or $B_2B_3B_7$. They suggested that two or more polygenic complexes, such as B_{Sm} and B_{Dm} (5), would provide excellent genetic backgrounds for obtaining immunity. Innes (17) reported that Arnold's upland Tanzania lines contained a polygenic complex that measurably enhanced the resistance of the B_2 gene. Innes postulated that because the resistance had been obtained during pedigree breeding, polygenes had been built (possibly by inversions) into an effective block resembling a single gene or closely linked genes, ie, a super-gene or an "effective factor" (21). The polygenic complex, B_{Sm}, is likely a component of Im 216.

Other Disease/Crop Complexes

We believe the principles successfully employed to develop long-term immunity to bacterial blight of cotton have application to other disease/crop complexes. We suggest the following approach (Fig. 10), with modifications depending on the crop and the disease.

A backcrossing program is commenced with one parent possessing two or more known single resistance genes and the other parent possessing polygenic resistance. The parent with polygenic resistance must be used as the recurrent parent in the backcrosses; otherwise, the polygenic resistance is likely to be lost through repeated backcrossing. A diallel crossing procedure would probably be useful to identify the most desirable resistance combination(s) for the initial cross(es). Segregating populations should be inoculated with a compatible race mixture of the pathogen that will attack all the known single resistance genes involved and, if available, combinations of the resistance genes. The most resistant plants are then selected to be used as parents for each additional cycle of backcrossing to the recurrent parent. If one or more of the resistance genes being transferred are recessive, a generation of selfing between each backcross is mandatory to allow the identification of plants with those genes. Once immunity has been attained, other methods of breeding (such as pedigree selection) can be employed. Final selections of cross-pollinated crops could be screened and inbred until homozygous, then intercrossed to restore vigor. Self-pollinated crops can be screened and line-pedigreed until homozygous.

Regardless of the degree of cross-pollination in the crop, pollination control, eg, selfing, is essential. Even in a self-pollinated crop, selfing is rarely complete, and *any* cross-pollination at all will retard the development of a homozygous immune strain or will result in a loss of uniformity after immunity has been attained.

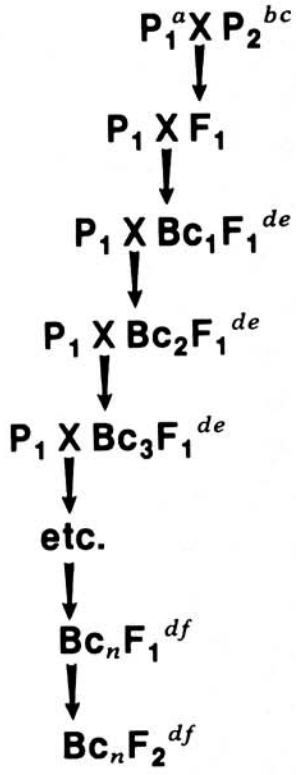

Fig. 10. Generalized backcrossing program to develop immunity in disease/crop complexes. *a* = Parent with polygenic resistance (recurrent parent). *b* = Parent with two or more dominant resistance genes (nonrecurrent parent). *c* = If one or more of the resistance genes in P_2 are recessive, a generation of selfing is required before inoculation, selection, and making each cross. *d* = Inoculate with a compatible race mixture and select those plants with the highest levels of resistance. *e* = Backcross (Bc) selected plants with P_1. *f* = Use individual plant-to-progeny row tests *with selfing* in every generation to detect homozygous immune lines.

The success of this approach (in effect the same as that advocated by Nelson [22]) depends greatly on the availability and use of pathogen genotypes that permit consistent and rigorous screening of plant genotypes in segregating generations. Ideally, plants chosen should possess the polygenic background plus one or more (preferably more) genes with large effects.

Conclusions

Immunity to bacterial blight of cotton was developed by combining several single-gene resistance factors onto a polygenic resistance background. The initial breeding procedure involved backcrossing, with the recurrent parent possessing the polygenic resistance; screening the segregating progeny after each backcross with a compatible mixture of virulent races of the pathogen; and selfing the selected plants. Pedigree breeding with continued screening and selfing was employed in later generations. In experiments subsequently conducted in many cotton-growing areas of the world, this immunity remained stable for over 20 years. The likelihood is advanced that similar breeding and screening procedures would prove useful for deriving long-term immunity in other disease/crop complexes.

Acknowledgments

We thank L. S. Bird of Texas A&M University and R. R. Nelson of Pennsylvania State University for the many useful suggestions made in their reviews of this manuscript.

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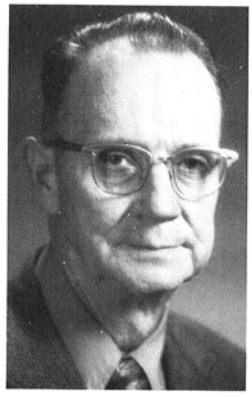

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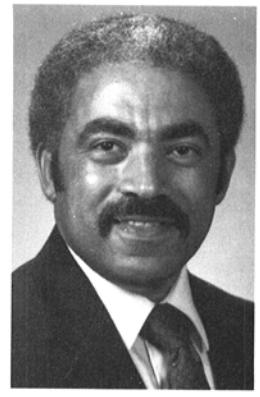

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