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BREEDING, GENETICS, & GENOMICS

Evaluation of Resistance to Tarnished Plant Bug in Small Plots of Cotton for More than Twenty Years

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ABSTRACT

Tarnished plant bug (TPB) (*Lygus lineolaris* Palisot de Beauvois; Hemiptera: Miridae), is the number one insect pest in U.S. Mid-South crops including cotton. Development and deployment of lines exhibiting partial resistance to TPB could reduce the quantity of insecticides needed to control this pest. We have evaluated TPB resistance of cotton lines in small plots since 2003. Insecticides were not applied for TPB. When TPB damage was readily seen in flowers of the susceptible check, examination for "dirty flowers" was made on six white flowers per day for 5 to 8 days. Cumulative percent dirty flowers over sampling days was then calculated for each plot. Over the last 21 years, we evaluated 2,724 lines in 102 different tests. Variation between supposed resistant and susceptible check cultivars were relatively consistent over tests. These data were used to quantify the level of TPB resistance in breeding strains and in 90 germplasm lines and cultivars released from the University of Arkansas Cotton Breeding Program, as well as cultivars and breeding lines in the annual Arkansas Cotton Variety Test and Regional Breeders' Network Test. Over the years, nectariless and highly pubescent lines have consistently provided some TPB resistance. However, TPB resistance has been found in some nectaried and glabrous lines. These findings suggest the existence of different mechanisms for TPB resistance.

Tarnished plant bug (TPB) (*Lygus lineolaris* Palisot de Beauvois; Hemiptera: Miridae) is the number one insect pest affecting U.S. Mid-south crops including cotton (*Gossypium hirsutum* L.) production (Cook and Threet, 2022). Successful elimination of the boll weevil (*Anthonomus grandis* Boheman) and widespread adoption of transgenic insecticidal cottons for control of the heliothine complex (bollworm [*Helicoverpa zea* (Boddie)] and tobacco budworm [*Heliothis virescens* (F.)]) have reduced insecticide applications and triggered increased injury and economic damage from TPB. TPB use needle-like piercing-sucking mouthparts to feed on developing squares and small bolls. As such, the TPB bypasses the endotoxins that are consumed by insects with chewing mouthparts (e.g., the heliothine complex).

Before the recent release of cultivars possessing ThryvOn® technology, TPB pest management programs relied almost exclusively on chemical control. However, there were considerable efforts by researchers to develop genetic lines of cotton that exhibited host plant resistance to TPB in the 1970s and 1980s. This effort primarily focused on morphological traits as reviewed by Jones (1972) and Jenkins (1982). The Frego-bract (twisted) trait has been associated with increased boll weevil resistance, but high susceptibility to TPB (Maredia et al., 1993). Traits found to be associated with resistance to TPB included nectariless, pubescent leaves and stems, high glanding (gossypol glands present in calyx crown), and normal leaf shape (relative to okra leaf). The nectariless characteristic showed the most consistent TPB resistance. Extrafloral nectaries found on veins of leaves and at the base of the floral buds attract beneficial insects but also encourage TPB populations, thus potentially increasing crop susceptibility.

The nectariless trait was derived from the wild tetraploid species of cotton, *G. tomentosum* Nuttall, which is native to Hawaii. Meyer and Meyer (1961) successfully transferred the trait to Upland cotton and showed that two recessive genes controlled its expression. In separate studies, no deleterious effects were associated with the nectariless trait (Meredith, 1980; Meredith et al. 1973). In a review, Meredith (1998a) cited 10 studies that reported reductions in

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Lygus spp. populations on nectariless cotton ranging from 26.2 to 66.6% with an average of 48.8%. He indicated that most studies measuring both target insects and their natural enemies showed a greater reduction in target insects than natural enemies. Benedict et al. (1981) reported that the genetic background of nectariless lines affected the mortality of adults and nymphs of western plant bug (*Lygus hesperus* Knight). This finding suggests that a resistance mechanism in addition to the nectariless trait might be present in some lines.

An Australian study, using plot sizes ranging from 0.25 to 10 ac, suggested that the benefit of nectariless trait lessened as plot size increased (Adjei-Maafo and Wilson, 1983). However, Scott et al. (1988) conducted a definitive study of 33 fields (over two years) ranging from 40 to 150 acres in the Mississippi River Delta and found reductions of greater than 40% in TPB populations in each of two years. They concluded that nectariless cotton can suppress plant bug populations over a large area of cotton. The development and release of nectariless cultivars by private companies made this large-plot study possible.

W. R. Meredith, Jr. was responsible for much of the foundational breeding work associated with the nectariless trait in cotton. His releases included nine nectariless germplasm lines (Meredith, 1977), eight isogenic lines having combinations of sub-okra leaf, smooth leaf, and nectariless trait (Meredith, 1998b), and a nectariless cultivar (Meredith, 1993). The University of Arkansas (UA) Cotton Breeding program has used several of these lines (specifically MD09ne, MD25ne, MD25-26ne, MD26ne, MD51ne, and nectariless isogenic lines of 'DES 119') as nectariless parents.

Nectariless cultivars listed with Plant Variety Protection (PVP) numbers include 'Stoneville 731N' (PVP 007600048), 'Stoneville 825' (PVP 007900024), 'Deltapine NSL' (PVP 008300112), and 'DP 0935 B2RF' (PVP 200900062). 'MD51ne' (PI 566941, Meredith, 1993) and 'UA212ne' (PI 692970, Bourland and Jones, 2020) were released as public cultivars without PVP. Various studies mention nectariless Acala, Coker (specifically Coker 84-828ne), and other Deltapine lines, but the release status of these lines is unclear.

Stoneville 825 was the most successful of the nectariless varieties. It occupied from 7 to 17% of U.S. acreage (18 to 45% of Arkansas acreage) in the early 1980s and from 9 down to 3% of U.S. acreage (19 down to 4% of Arkansas acreage) in the late 1980s. Before boll weevil eradication and the advent of Bt cotton, the full impact of the nectariless trait on TPB was hindered by systematic treating of fields for boll weevils and the heliothine (bollworm/budworm) complex. After boll weevils were essentially eradicated and the heliothine complex was mostly controlled by Bt cotton, the relative importance of TPB as a cotton pest greatly increased. The late maturity of DP 0935 B2RF, a nectariless, Bt cultivar, might have hindered its acceptance and popularity.

Resistance to TPB is usually not associated with the glabrous plant trait (Jenkins, 1982; Meredith and Schuster, 1979). Arkot 8110 (Bourland et al., 1997) is a germplasm line released by UA, which possesses glabrous leaves and stems. Using the techniques of Maredia et al. (1994), Arkot 8110 was found to be as resistant as DES 119 (a pubescent cultivar) and Stoneville 825 (a pubescent, nectariless cultivar) in two field tests conducted in 1988 and 1994. These results suggest that different sources of resistance to TPB exist. If such sources are additive to the role of the nectariless trait, TPB resistance might be enhanced by combining undefined sources of resistance into nectariless lines.

In seedling cotton, the apical meristem is the preferred feeding site of TPB. As cotton develops, TPB will feed on floral buds (squares). Small squares that have been fed upon will usually shed from the plant. Before the anthers are easily visible, TPBs feed on the totality of the floral buds, causing them to shed. As the square grows, the anthers reach a sufficient size for TPB to feed on individual pollen sacks (Pack and Tugwell, 1976). When feeding is localized to the anthers, a square rarely sheds, but the injury can be seen as discoloration and anther damage when the flower opens. Such flowers often are referred to as dirty blooms or dirty flowers.

Historically, the principal method used to assess TPB resistance of cotton cultivars has been to compare yields of lines in insecticide-free plots versus yields in sprayed plots. Maredia et al (1994) developed a method of evaluating TPB by slicing squares and examining damage to anthers. Examination of anthers in squares rather than in flowers was required to distinguish between boll weevil and TPB injury. However, without boll weevils present, TPB damage to lines can be evaluated by examining the presence and degree of darkened anthers in white flowers (day of anthesis). Examining white flowers requires much less time than slicing squares.

The objective here is to review methods and summarize results from the small-plot tests that were used to evaluate resistance to TPB in cotton lines at Keiser, AR, from 2003 through 2023.

MATERIALS AND METHODS

Since 2003, response of cotton genotypes to TPB has been evaluated annually in small plots in one of two adjacent fields at the Northeast Research and Extension Center at Keiser, AR, on Sharkey clay (very fine, smectitic, thermic Chromic Epiaquert). The test plots were managed using standard production practices except that no insecticides were applied to control TPB. In 2003 to 2005, plots were 2 rows replicated six times; in 2006 to 2012, plots were 1 row replicated 12 times; in 2013 to 2023 plots were 1 row replicated eight times. Each year, plots were arranged vertically down the rows with 4-row strips of a highly susceptible line (Frego-bract) planted between tests. Each plot within a replication was then an equal distance from the Frego-bract strips. Frego-bract lines have long been associated with TPB susceptibility (Jones, 1972). An early-maturing Frego-bract line designated as RBCDHGPIH-1-97 from the Texas A&M MAR program (or a closely related derivative) was used as the highly susceptible check in every test.

In early years, we attempted to plant the Frego-bract strips 2 to 3 wk prior to the tests. TPB were expected to build-up in the Frego-bract strips then bleed into the tests as the Frego-bract strips matured. This differential planting date strategy was difficult to achieve in many years (due to weather) and contributed to increased weed control issues (due to variation in herbicide programs). In recent years, the strips and tests have been planted on the same day. In some years, the strips were mowed as TPB populations increased, which caused TPB to move into the adjacent test plots. Plantings usually occurred mid- to late May, but wet weather sometimes caused planting to be delayed until June (Table 1).

Table 1. Planting dates, number of tests, and number of entries (including checks) in Arkansas Strain tests, Arkansas Cotton Variety tests, and Regional Strain tests evaluated for response to TPB from 2003 through 2023 at Keiser, AR

	Planting date		Ark. Strain Tests		Ark. Variety Tests		Reg. Strain Tests		All tests			
Year	Gregorian	Julian	No. tests	Entries tested	No. tests	Entries tested	No. tests	Entries tested	No. tests	Entries tested	Dirty flowers (%)	Avg. R^2
2003	14 Jun	165	1	22					1	22	58.0	52.9
2004	21 May	142	2	44					2	44	42.5	78.0
2005	21 May	141	2	44					2	44	38.0	83.9
2006	17 May	137	2	44	3	84	1	25	6	153	30.8	70.7
2007	11 May	131	2	44	2	79	2	58	6	181	40.2	69.8
2008	23 May	144	2	44	2	54	2	57	6	155	30.1	66.1
2009	22 May	142	2	44	2	58	3	80	7	182	49.4	63.3
2010	28 May	148	2	44	2	59	2	60	6	163	43.3	65.6
2011	31 May	151	2	44	2	52	2	58	6	154	51.7	60.1
2012	29 May	150	2	44	2	44	2	57	6	145	57.7	50.2
2013	14 Jun	165	2	44	1	44	2	58	5	146	62.8	21.7
2014	23 May	143	2	44	1	44	1	31	4	110	51.0	44.3
2015	14 Jun	165	2	44	1	44	1	30	4	118	75.6	54.2
2016	11 May	132	2	44	2	48	1	30	5	122	53.8	64.9
2017	15 May	135	2	44	2	62	1	36	5	142	46.8	73.3
2018	29 May	149	2	44	3	85	1	26	6	155	46.5	60.9
2019	3 Jun	154	2	44	2	69	1	26	5	131	67.2	45.4
2020	21 May	142	2	44	2	66	1	18	5	128	70.4	66.4
2021	20 May	140	2	44	2	64	1	30	5	138	77.3	50.5
2022	12 May	133	2	44	2	64	1	36	5	144	51.3	66.1
2023	18 May	138	2	44	2	60	1	26	5	130	65.9	49.2
Sum			41	902	35	1080	26	742	102	2724		

Over years, we evaluated 2,724 entries in 102 different tests for response to TPB (Table 1). Entries in each test included one or more Frego bract checks (highly susceptible). Many normal bract lines were evaluated for multiple years and/or in multiple tests per year. Over the 21 years, approximately 1,500 different genotypes were evaluated for response to TPB in these tests. Each year, entries in Arkansas Advanced Strain Test and Arkansas New Strain Test (except 2003) were evaluated and included a moderately resistant check (PSC 355, 2003-2005; DP 393, 2006-2015; and UA222, 2016-2023), a moderately susceptible check (SG 105, 2003-2010; UA48, 2011-2023) and the Frego-bract checks. Evaluation of entries in the Arkansas Cotton Variety Test and the Regional High-Quality Strain (RHQ) Test was initiated in 2006, and the Regional Breeders' Network Test (RBTN) was added in 2007. All RBTN tests included a moderately resistant check (DP 393) and a moderately susceptible check (FM 958). Because most RHQ entries were duplicated in other tests, the RHQ Test has not been evaluated since 2013.

Each test included one to three Frego-bract (susceptible check) entries. When TPB damage could be seen readily in white flowers of the susceptible check, evaluation of dirty flowers was initiated. In 2003 to 2005, up to 10 white flowers per plot per day were examined and rated as 0 (no darkened anthers), light (up to 50% darkened anthers, average of approximately 30%), or heavy (more than 50% darkened anthers, average of approximately 80%) TPB damage (Fig. 1). Cumulative counts over 5 d were calculated as:

% dirty flowers = (no. light + no. heavy) / no. flowers) *100, and

% darkened anthers = ((no. light * 0.3) + (no. heavy * 0.8)) / no. flowers) *100.

Coinciding with the change in plot size and number of replications, sampling procedures were modified in 2006. Since then, up to six white flowers per plot per day have been examined for the presence or absence of any darkened anthers. Cumulative counts over 5 to 6 sample days were used to determine percent dirty flowers.

The Frego-bract checks and other highly susceptible lines had a scarcity of white flowers in the 2021 through 2023 tests. However, final boll numbers differed among plots with some plots approaching normal boll productions. To access the boll load variation in the plots, the plots were evaluated with the visual rating scale normally used in our progeny rows evaluations. After defoliation and boll opening, each plot was visually rated from 0 (no bolls) to 10 (excellent boll load).

Figure 1. Dirty flowers associated with tarnished plant bug (TPB) damage: A. Normal shaped bract flower with a TPB nymph and less than 50% damaged anthers; B. Frego-bract flower with more than 50% damaged anthers.

The normal-bract lines in the TPB tests also were evaluated annually in Arkansas strain tests and Arkansas Cotton Variety tests, which are typically evaluated at four or five sites in Arkansas each year. Measurements of yield, maturity, and fiber quality are collected at each site and averaged over sites. At one location (typically Keiser), plant hairiness of lines is characterized by visually rating leaf and stem pubescence (Bourland et al., 2003), and by counting bract trichomes under a viewing scope (Bourland and Hornbeck, 2007). In each year, Pearson's simple correlation coefficients were calculated between percent dirty flowers (for non-Frego bract lines in each TPB test) with lint yield and percent open bolls (averaged across locations) and with the three plant hairiness variables.

RESULTS AND DISCUSSION

Percent Dirty Flowers vs. Planting Date. In most years, boll set varied greatly between plots with the most resistant lines approaching normal boll load (Fig. 2a). Tests within years typically had similar average percent dirty flowers, coefficient of determinations (R^2), and coefficient of variations (data not shown). Average R^2s associated with percent dirty flowers were less than 50% over tests in only four of the 21 years (2013, 2014, 2019, and 2023) (Fig. 3). The 2013 season, which had the lowest R^2s, was highly troublesome at Keiser. Wet conditions delayed planting of all tests. Planting of cotton cultivar, strain, and breeding tests were delayed until the last few days of May. Demand for the plot planter and subsequent rains delayed planting of the TPB tests until 14 June. The plots achieved good stands, but plant development was subsequently slowed by cloudy conditions. Measurable rainfall was received on 12 of 14 d from 31 July to 13 August. Under these conditions, extremely high TPB populations were experienced. Consequently, most lines in the TPB tests produced few flowers and subsequent plants appeared to be mechanically stripped after defoliation (Fig. 2b). Total flowers per plot over the six sample

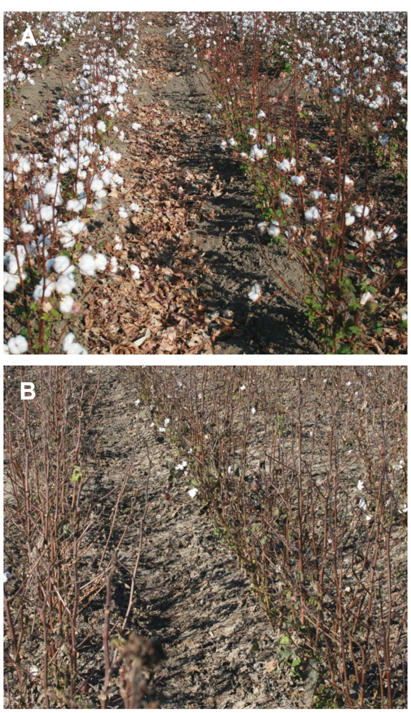

Figure 2. Variation in severity of damage in tarnished plant bug tests: A. Typical variation found between resistant and susceptible cotton lines (picture taken in 2010); B. Severe damage overwhelmed all lines in 2013.

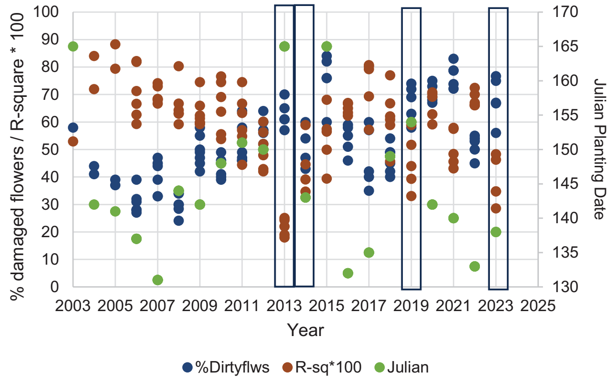

Figure 3. Mean % damaged flowers and R-squares (*100) by Julian planting dates associated with small plot tarnished plant bug tests at Keiser, AR, in 2003 through 2023. Average R-squares for years marked with rectangles were less than 50%.

dates in 2013 ranged from 13 to less than 1 (data not shown). Low flower production was also noted in the 2014, 2019, and 2023 tests, particularly on the susceptible Frego-bract check. In these years, high TPB populations apparently overwhelmed square and flower development.

From 2004 through 2010, percent dirty flowers values were always less than 50% and R^2s exceeded 63% (Table 1). In the 13 yr since 2010, except 2017 and 2018, percent dirty flowers exceeded 50% and R^2s tended to be lower than the earlier years. These data suggest that percent dirty flowers has increased in recent years, presumably a reflection of recent increases in TPB populations. It is doubtful that the change in testing 12 to 8 replications in 2013 contributed to the higher percent dirty flowers. Higher TPB populations and damages in recent years could be related to increased problems with controlling resistant pigweed, an alternative host of TPB, in the plots.

As planting date was delayed, percent dirty flowers tended to increase, and R^2s associated with tests tended to decline (Fig. 4). If June plantings were discarded, the relation of planting date to percent dirty flowers was essentially neutralized ($y = 0.09 + 37.65$, $R^2 = 0.002$), and the R^2s associated with the tests were reduced ($y = -0.45 +127.12$, $R^2 = 0.056$). Coincidentally, the tests in 2003, 2013, and 2015 were all planted on 14 June (Table 1). Of all 102 tests, 25% had R^2s of less than 50%, and 53% had R^2s greater than 60% (Table 2). Tests having R^2s greater than 60% tended to have earlier planting dates and lower percent dirty flowers than the other tests.

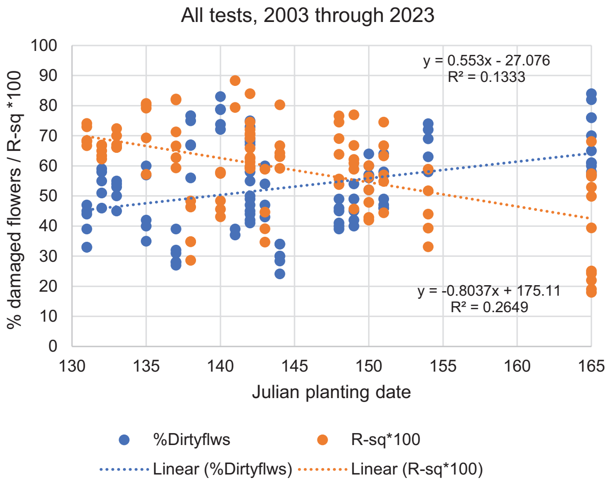

Figure 4. Effects of planting date (Julian date) on % damaged flowers and R-squares in 102 tarnished plant bug tests conducted at Keiser, AR, in 2003 through 2023.

Obtaining optimum sequencing of flower production and TPB populations has been difficult to achieve and will likely always be challenging. Plants in early plantings might escape damage from high TPB populations, whereas plants in later plantings might be overwhelmed by TPB. Over 21 yr, these TPB tests were planted after all other cotton tests were planted on the station in hopes that onset of flowering would be synchronized with high TPB populations. In retrospect, earlier planting dates could have achieved better results, but wet spring conditions compounded by the clay soil frequently prevented earlier planting.

Percent Dirty Flowers vs. Percent Darkened Anthers. In 2003 through 2005, flowers from each plot were differentiated as to whether they had less than or more than 50% discolored anthers (Fig. 1). From these data, percent dirty flowers (percent of flowers having any discolored anthers) and percent darkened anthers (average percent of discolored anthers in all flowers) were determined. Over 3 yr, percent dirty flowers and percent darkened anthers were found to be highly correlated ($r \geq 0.95$) in each of five tests (data not shown). Differentiating flowers by the degree of darkened anthers required more time and was more subjective than simply determining if flowers had any darkened anthers. The degree of anther darkening on an individual flower could be related to the age of the developing bud when TPB feeding occurs. Estimating percent darkened anthers was terminated after the 2005 season.

Percent Dirty Flowers vs. Boll Load Ratings. If high TPB populations occurred before uniform flower initiation, flower production was frequently scarce, particularly in highly susceptible lines. Scarcity of white flowers hinders the attainment of reliable percent dirty flowers data. To obtain genotypic differences in these situations, visual ratings of final boll load might provide differentiation of lines. In 2021 through 2023, boll load in the plots were visually rated using a scale of 0 (no bolls) to 10 (excellent boll load). In our progeny row tests, these visual ratings normally range from 3 to 8 (rarely 9). In these TPB tests, ratings ranged from 0 to 7. The boll load ratings were highly correlated with percent dirty flowers in 14 of the 15 tests conducted in 2021 through 2023 (Table 3). The lowest correlation (non-significant) value was in the 2023 New Strain Test, which also had a low (28.6%) R^2 associated with percent dirty flowers.

The R^2s associated with visual boll load ratings were higher than those associated with percent dirty flowers in the 10 tests conducted in 2021 and 2023 and were similar in the five tests conducted in 2022

Table 2. Average planting date, R^2s, and percent dirty flowers associated with ranges of R^2s in small plot tarnished plant bug tests at Keiser, AR, from 2003 through 2023

R^2 range	No. tests	Avg. plant date	Avg. R^2	Avg. dirty flowers
----- % -----		------ Julian ------	---------- % ---------	---------- % ---------
0 to 100	102	144.4 ± 9.6	59.0 ± 15.0	52.8 ± 14.5
0 to <30	6	160.5 ± 11.0	22.8 ± 4.0	65.1 ± 7.2
30 to <40	7	147.9 ± 10.1	36.5 ± 2.7	67.3 ± 8.9
40 to <50	13	146.8 ± 7.9	45.6 ± 2.4	64.3 ± 11.7
50 to <60	22	148.0 ± 8.6	57.0 ± 2.5	56.4 ± 14.9
60 to <70	33	140.5 ± 8.4	65.2 ± 2.6	46.7 ± 12.0
70 to <80	15	140.3 ± 6.8	74.0 ± 3.0	47.2 ± 9.6
>80	7	138.7 ± 3.3	82.5 ± 2.7	36.0 ± 10.3

Table 3. Simple correlation (r) of percent dirty flowers and boll load ratings in tarnished plant bug tests conducted at Keiser, AR, in 2021 through 2023

Test	2021		2022		2023	
	No.	r	No.	r	No.	r
Ark. Transgenic Variety Test	46	-0.654**z	42	-0.569**	46	-0.712**
Ark. Conventional Variety Test	16	-0.849**	22	-0.866**	14	-0.736**
Regional Breeders' Network Test	28	-0.810**	36	-0.556**	26	-0.648**
Ark. Advanced Strain Test	22	-0.912**	22	-0.664**	22	-0.708**
Ark. New Strain Test	22	-0.811**	22	-0.735**	22	-0.137 ns

z ** Differs from 0 at 0.01 probability level.

(Table 4). Higher R^2s indicate that more variation was associated with specified sources of variation and less to random error. Boll load ratings can be obtained with much less time and labor than percent dirty flowers. Also, boll load ratings are less affected by the timing of TPB and flowering than is percent dirty flowers.

Percent Dirty Flowers in Frego-Bract vs. Normal-Bract Lines. Frego-bract (Fig. 1) lines were included as checks in all 102 tests conducted since 2003. Strains and cultivars having normally shaped bracts have produced consistently lower percent dirty flowers than the susceptible Frego-bract checks. Out of 2,724 normal-bract lines evaluated,

Table 4. Mean visual boll load ratings and percent dirty flowers of check cultivars in tarnished plant bug tests of entries in the Arkansas Transgenic Variety Test (Trans. Variety), Arkansas Conventional Variety Test (Conv. Variety), Advanced Strain Test, New Strain Test, and Regional Breeders' Testing Network Test (RBTN) at Keiser, AR, from 2021 to 2023

	Dirty flowers (%)				Visual boll load ratings			
Test	2021	2022	2023	Mean	2021	2022	2023	Mean
Trans. Variety								
Normal bract	71	43	56	57	3.3	4.5	4.7	4.2
Frego-bract check	98	89	73	87	0.1	2.6	1.6	1.4
LSD 0.10	9	7	9		0.7	0.6	0.6	
R^2 (x 100)	45.6	66.1	48.3	53.3	70.1	53.6	77.5	67.1
Conv. Variety								
UA222	76	40	73	63	3.4	4.7	3.3	3.8
UA48	82	59	72	71	1.4	3.8	1.6	2.3
Normal bract	77	49	73	66	3.0	4.5	2.9	3.5
Frego-bract check	97	91	86	91	0.1	2.6	1.7	1.5
LSD 0.10	8	8	10		0.5	0.6	0.8	
R^2 (x 100)	48.4	72.4	46.3	55.7	84.5	53.6	42.6	60.2
Advanced Strain								
UA222	72	38	61	57	3.9	4.8	3.8	4.2
UA48	87	55	69	70	1.4	4.8	3.1	3.1
Normal bract	77	50	65	64	3.5	4.6	3.7	3.9
Frego-bract check	98	90	88	92	0.1	2.7	1.8	1.5
LSD 0.10	7	9	11		0.7	0.6	0.7	
R^2 (x 100)	57.9	67.0	34.8	53.2	78.5	58.7	53.7	63.6
New Strain								
UA222	90	40	63	64	3.7	4.3	2.8	3.6
UA48	84	60	74	73	1.1	3.4	3.8	2.8

Table 4. continued

	Dirty flowers (%)				Visual boll load ratings			
Test	**2021**	**2022**	**2023**	**Mean**	**2021**	**2022**	**2023**	**Mean**
Normal bract	**81**	**46**	**75**	**67**	**3.4**	**4.2**	**3.3**	**3.6**
Frego-bract check	**99**	**91**	**89**	**93**	**0.2**	**2.3**	**2.5**	**1.7**
LSD 0.10	**6**	**9**	**10**		**0.7**	**0.6**	**0.8**	
R^2 (x 100)	**57.5**	**66.1**	**28.6**	**50.7**	**80.6**	**64.9**	**31.3**	**58.9**
RBTN								
DP 393	**66**	**50**	**46**	**51**	**4.5**	**5.2**	**3.9**	**4.5**
UA222	**73**	**46**	**43**	**54**	**3.8**	**5.4**	**4.5**	**4.6**
FM 958	**71**	**55**	**55**	**60**	**2.3**	**3.7**	**3.1**	**3.0**
Normal bract	**72**	**51**	**53**	**59**	**2.9**	**4.4**	**3.4**	**3.6**
Frego-bract check	**100**	**87**	**88**	**92**	**0.1**	**2.3**	**1.4**	**1.3**
LSD 0.10	**9**	**7**	**11**		**0.6**	**0.6**	**0.7**	
R^2 (x 100)	**43.1**	**74.0**	**19.0**	**45.4**	**72.5**	**65.8**	**53.7**	**64.0**

only 88 produced percent dirty flowers equal to the Frego-bract checks (data not shown). Only 11 of these 88 lines occurred in tests that had $R^2 > 40\%$ for percent dirty flowers, and 9 of these 11 occurred in the 2023 Arkansas Cotton Variety tests. Thus, when tests with $R^2 < 40\%$ and the 2023 variety tests were discarded, only 2 out of 2,402 observations of normal-bract lines produced percent dirty flowers equal to the Frego-bract checks. None of the normal-bract lines had greater percent dirty flowers than the Frego-bract checks.

Compared to normal bract entries in 2021 through 2023 tests, Frego-bract checks averaged 28% higher dirty flowers and 2.2 lower boll load ratings (Table 4). These differences were consistent over the 15 tests. Thus, these tests confirm that Frego-bract lines are highly susceptible to TPB.

Percent Dirty Flowers in Nectariless vs. Nectaried Lines. Increased resistance to TPB in nectariless lines is well documented (Jenkins, 1982; Jones, 1972). Both nectariless and nectaried lines were included in 44 of these TPB tests over the years (Table 5). The 114 nectariless lines in these tests averaged 48.8% dirty flowers compared to 53.2% for the 813 nectaried lines. Response of nectariless and nectaried lines to TPB were similar in both Arkansas Strain tests and Regional tests. Nectariless lines have shown consistently good resistance, but generally not more resistance than the best nectaried lines. This suggests that variation in resistance to TPB exists in highly adapted genetic lines, and that more than one source of resistance could be operative because resistance was not restricted to the nectariless lines. In some years, high TPB densities likely

Table 5. Average percent dirty flowers associated with nectaried (NE), nectariless (ne), transgenic (TG), and conventional (CV) lines when evaluated in same test

Factor	Strain tests	Regional tests	Variety tests	All tests
No. of tests with both NE and ne lines	**24**	**20**	**0**	**44**
No. of NE lines used	**397**	**416**	**0**	**813**
% Dirty flowers on NE lines	**53.9**	**52.4**	**0**	**53.2**
No. of ne lines used	**79**	**35**	**0**	**114**
% Dirty flowers on ne lines	**50.0**	**47.3**	**0**	**48.8**
No. of tests with both TG and CV lines	**0**	**8**	**17**	**25**
No. of TG lines used	**0**	**93**	**378**	**471**
% Dirty flowers on TG lines	**0**	**47.2**	**41.9**	**43.6**
No. of CV lines used	**0**	**73**	**85**	**158**
% Dirty flowers on CV lines	**0**	**46.7**	**45.8**	**46.1**

overwhelmed resistance or non-preference inferred by the nectariless trait in these small plots.

Percent Dirty Flowers in Transgenic vs Conventional Lines. Transgenic and conventional lines were evaluated together in the RHQ tests (2006–2013) and in Arkansas Cotton Variety tests (2005–2014) for a total of 25 tests that included 471 transgenic lines and 158 conventional lines (Table 5). Compared to conventional lines, the transgenic lines produced lower percent dirty flowers in the cultivar tests, but slightly higher percent dirty flowers in the RHQ tests, which suggest that most transgenes do not have strong effects on percent dirty flowers.

Relationship with TPB resistance associated with ThryvOn technology (B3TXF) appears to differ from previously tested transgenes. Before 2023, cultivars possessing ThryvOn technology had not been evaluated in Arkansas Cotton Variety Test or in our TPB tests. Out of 44 cultivars in the 2023 test, the 10 B3TXF cultivars performed well relative to visual boll load rating (9 of the top 10 cultivars) and percent dirty flowers (8 of the lowest 10 cultivars) (Bourland et al., 2024). These findings were impressive, although they are based on 1 yr of data.

Relationship of Percent Dirty Flowers to Yield and Earliness. Relationships of lint yield to percent dirty flowers varied greatly over tests (Table 6). Correlation coefficients of lint yield with percent dirty flowers differed from zero in only 13 of 78 tests with nearly equal number of tests having negative and positive relationships. Compared to the relationship with lint yield, the relationship of percent open bolls to percent dirty flowers was more directional with a tendency for higher percent open bolls (i.e., earlier maturity) being related to increased percent dirty flowers (i.e., lower TPB resistance). Both lint yield and percent open bolls data represent means over all test locations and represent the general tendency of the lines. Relationships of percent dirty flowers to lint yield and earliness could be affected by the timing and density of TPB populations.

Relationship of Percent Dirty Flowers to Plant Hairiness. Increased leaf pubescence exhibited a stronger association with TPB resistance (lower percent dirty flowers) than the other two plant hairiness parameters (Table 6). Thus, TPB resistance tended to be higher on hairy leaf lines than on smooth leaf lines. Also, higher bract trichome density, and to lesser extent increased stem pubescence, were related to lower percent dirty flowers. In his review, Jenkins (1979) noted that increased leaf pubescence was related to increased TPB resistance.

Variation in Percent Dirty Flowers Among Check Cultivars. Over the 44 Arkansas Cotton Variety tests and RHQ tests, percent dirty flowers for the Frego bract (85.1%) far exceeded the mean percent dirty flowers for non-Frego bract entries (48.7%). Similarly, percent dirty flowers were higher in the Frego-bract checks than the normal shaped bract checks in the New Strain Test (Fig. 5A), the Advanced Strain Test (Figure 5B), and the RBTN Test (Fig. 6). Both the moderately resistant and

Table 6. Relationships of percent dirty flowers with yield and morphological traits in 102 small-plot tarnished plant bug (TPB) tests at Keiser, AR, 2003 through 2023

	Simple correlations[z] of percent dirty flowersy with:				
Factor	**Lint yield[x]**	**% Open bolls[x]**	**Leaf pubescence[w]**	**Stem pubescence[w]**	**Bract trichomes[w]**
No. tests	**78**	**77**	**77**	**63**	**73**
Mean correlation coef.	**-0.053**	**0.100**	**-0.190**	**-0.073**	**-0.126**
Max. correlation coef.	**0.464**	**0.646**	**0.332**	**0.580**	**0.402**
No. coefficients > zero	**5**	**11**	**0**	**1**	**2**
Min. correlation coef.	**-0.607**	**-0.502**	**-0.695**	**-0.433**	**-0.684**
No. coefficients < zero	**8**	**3**	**19**	**2**	**9**

[z]Correlations from 25 tests that had R^2s < 50% for % dirty flowers were not included in comparisons. Open bolls (%) and pubescence data were not available in all tests.

[y]Response to TPB determined by % dirty flowers in tests not treated for TPBs.

[x]Data for lint yield and % open bolls were means over all Arkansas locations for variety and strain tests (data not shown).

[w]Leaf and stem pubescence were visually rated as 1) smooth, 3) light hairs, 5) hairy or 7) very hairy and marginal trichome density on bract samples were counted in tests receiving standard TPB control at Keiser, AR (data not shown).

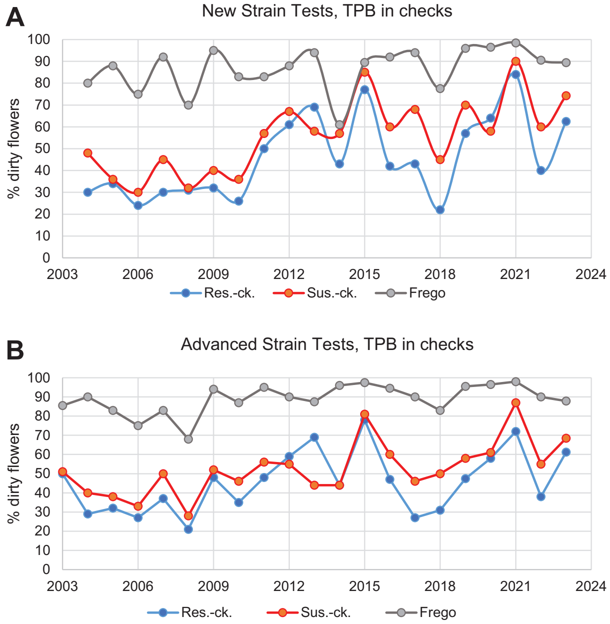

Figure 5. Dirty flowers (%) associated with check cultivars in the Arkansas New Strain (A) and Advanced Strain (B) tests. Partially resistant checks (Res-ck.) were PSC 355 (2003-2005), DP 393 (2006-2015), and UA222 (2016-2023). Relatively susceptible checks (Sus.-ck.) were SG 105 (2003-2010) and UA48 (2011-2023). A Frego-bract line was used as a highly susceptible check in each test, all other checks had normal shaped bracts.

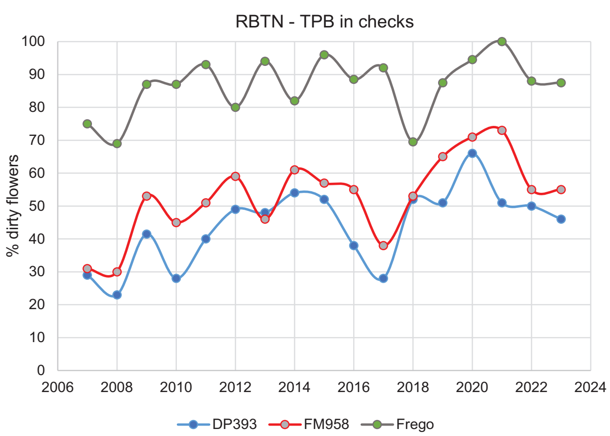

Figure 6. Dirty flowers (%) of check cultivars in the Regional Breeders' Network Test (RBTN) from 2007 through 2023. DP 393 showed more partial resistance than FM 958. A Frego-bract line was used as a highly susceptible check in each year.

moderately susceptible check cultivars had lower percent dirty flowers than the Frego-bract check in all tests except the 2014 and 2015 New Strain tests.

In most years, percent dirty flowers differed between the moderately resistant and moderately susceptible checks (Figs. 5 and 6). Variation between these checks was discernable in most years. Over years, percent dirty flowers for the moderately resistant and susceptible checks averaged 46 and 56%, respectively, in the New Strain tests; 46 and 53%, respectively, in the Advanced Strain tests; and 40 and 44%, respectively, in the RBTN Test. The relatively consistent performance of these check cultivars increases confidence in the data associated with the other lines.

TPB Resistant in UA Strains. In the UA Cotton Breeding Program, 72 Preliminary Strains were evaluated in replicated tests at four locations each year (Bourland, 2004, 2013). The best 18 of the Preliminary Strains were promoted and evaluated as New Strains for 1 yr. Superior New Strains were promoted and evaluated as Advanced Strains for 1 or 2 yr. New and Advanced Strains that appear to be highly susceptible to TPB can be discarded, but no direct selection for TPB resistance has been made.

Since 2003, a total of 360 different New Strains have been evaluated for resistance to TPB. Approximately half of these were promoted to Advanced Strains and evaluated in multiple years for a total of 735 observations of strains (Table 7). From these 735 observations, 479 (65.2%) and 678 (92.2%) were more resistant or equal to the moderately resistant and moderately susceptible check, respectively. For most strains, TPB data were collected for at least 3 yr and were used to determine release decisions. The Advanced Strains included 90 strains released as germplasm lines or cultivars with accompanying TPB data (data not shown). Out of the 90 released lines, 9, 42, and 39 were more, equal, and less resistant, respectively, than the moderately resistance checks; and 39, 46, and 5 were more, equal, and less resistant, respectively, than the moderately susceptible checks. Thus, 57 and 94% of the 90 released lines were equal to or more resistant than the moderately resistant and moderately susceptible lines, respectively. All were more resistant than the highly susceptible Frego-bract checks.

The nine lines that were more resistant than the moderately resistant check (as well as the other check cultivars) included: Arkot 9605ne (Bourland and Jones, 2006), Arkot 9631ne (Bourland and Jones, 2006), Arkot JJ46 (Bourland and Jones, 2008), Arkot 9623 (Bourland and Jones, 2009a), Arkot 9706 (Bourland and Jones, 2009b), Arkot 9815 (Bourland and Jones, 2010), Arkot 0403ne (Bourland and Jones, 2015), Arkot 0611 (Bourland et al., 2019), and UA212ne (Bourland and Jones, 2020). Four

Table 7. Number of entries that expressed more and equal percent dirty flowers as a moderately resistant and moderately susceptible check in tarnished plant bug evaluations of entries in Arkansas strain and Arkansas Cotton Variety tests from 2003–2023[z]

	---------- Arkansas Strain Tests ----------					--------------------- Arkansas Variety Tests -------------------				
		vs. Res. check		vs. Sus. check			vs. Res. check		vs. Sus. check	
Year	Total	More	Equal	More	Equal	Total	More	Equal	More	Equal
2003	18	0	13	0	13					
2004	36	0	20	12	24					
2005	36	1	31	11	25					
2006	36	11	20	22	11	78	7	55	36	42
2007	36	1	14	20	15	75	0	12	12	62
2008	36	2	27	6	28	50	12	34	33	17
2009	36	0	29	1	33	53	3	43	17	35
2010	36	0	10	8	26	55	0	29	6	40
2011	36	3	15	11	23	48	10	37	37	11
2012	36	5	30	9	23	40	10	30	21	19
2013	36	6	30	1	35	41	0	41	0	41
2014	36	4	32	1	19	40	1	31	5	38
2015	36	7	20	17	19	42	1	34	7	34
2016	36	1	12	14	22	45	2	27	29	16
2017	36	0	13	25	10	57	13	28	41	13
2018	36	0	4	10	25	80	2	51	34	46
2019	36	0	15	0	25	65	0	41	12	51
2020	36	3	15	10	17	61	3	30	10	37
2021	36	8	22	24	12	60	14	42	40	20
2022	36	0	19	23	13	60	3	43	46	13
2023	36	0	36	0	35	56	24	28	28	20
Sum	735	52	427	225	453	1006	105	636	414	555

[z]Moderately resistant checks were PSC 355 (2003-2006), DP 393 (2007-2015), and UA222 (2016-2023). Moderately susceptible checks were SG 105 (2003-2010) and UA48 (2011-2023). Entries in Arkansas Cotton Variety tests were compared to the means of moderately resistant and susceptible checks determined in adjacent strain tests.

of these nine resistant lines possess the nectariless trait. A total of 10 nectariless lines were among the 90 released lines evaluated for TPB resistance. The other six nectariless lines were either more resistant or equal to the moderately susceptible check. These observations suggest that resistance to TPB is available in some of these lines. However, direct selection for TPB resistance has not been made in this program.

TPB Resistant in Cotton Cultivars. Cotton lines entered in the Arkansas Cotton Variety Test have been annually evaluated for resistance to TPB since 2006 (Table 1). Results of these annual tests are available at www.ArkansasVarietyTesting.com (under “Cotton Reports”). Comparison of these cultivars over years is difficult because the same lines are not evaluated each year, and the TPB intensity differs across years as indicated by variation in percent dirty flowers (Fig. 1). In each year, all entries in the Cotton Variety tests were more resistant than the highly susceptible Frego-bract checks included in each test (data not shown).

A total of 1,006 observations of TPB evaluations in Arkansas Cotton Variety tests have been conducted since 2006 (Table 7). In contrast to the strain tests, the cultivar tests did not include designated moderately resistant and moderately susceptible entries for uniform comparisons across years. However, entries in the cultivar tests were compared to the means of the moderately resistant and moderately susceptible checks in the adjacent strain tests. From these 1,006 observations, 741 (73.7%) and 969 (96.3%) were

Table 8. Response (2019 through 2023) of returning entries in the 2023 Arkansas Transgenic Cotton Variety Test to tarnished plant bug[z] expressed as percent dirty flowers (DF) and as a percentile[y] (Pctl) of number of entries at Keiser, AR. Ranks (rk) within years represent relative DF of all entries (non-returning, returning, and new) in each test

	---- Over years ----				----- 2023 -----			----- 2022 -----			----- 2021 -----			----- 2020 -----			----- 2019 -----		
Variety	DF	*rk*	Pctl	*rk*	DF	*rk*	Pctl	DF	*rk*	Pctl	DF	*rk*	Pctl	DF	*rk*	Pctl	DF	*rk*	Pctl
	%				%			%			%			%			%		
DP 2239 B3XF	43	*1*	19	1	53	*15*	33	33	*2*	5									
PHY 360 W3FE	51	*4*	20	2	56	*25*	54	39	*12*	29	54	*1*	2	55	*6*	11	49	*2*	4
PHY 332 W3FE	54	*9*	21	3	52	*13*	28	38	*6*	14	71	*18*	39	54	*4*	7	56	*9*	17
NG 4190 B3XF	50	*3*	23	4	56	*26*	57	34	*3*	7	60	*3*	7						
NG 3195 B3XF	54	*8*	28	5	60	*31*	67	39	*8*	19	63	*7*	15	55	*5*	9			
PHY 415 W3FE	52	*7*	29	6	52	*12*	26	41	*17*	40	64	*10*	22						
ST 4595 B3XF	52	*6*	31	7	55	*24*	52	39	*9*	21	63	*9*	20						
PHY 400 W3FE	55	*10*	33	8	62	*38*	83	42	*20*	48	60	*4*	9	61	*11*	20	52	*4*	8
PHY 411 W3FE	58	*11*	40	9	60	*33*	72	37	*5*	12	71	*20*	43	65	*18*	33			
DG 3528 B3XF	48	*2*	42	10	54	*22*	48	41	*15*	36									
DP 1646 B2XF	59	*12*	45	11	57	*28*	61	42	*19*	45	77	*34*	74	64	*16*	30	55	*7*	13
PHY 443 W3FE	60	*13*	49	12	61	*34*	74	43	*21*	50	71	*19*	41	65	*17*	31	61	*25*	48
DP 2127 B3XF	61	*14*	56	13	61	*36*	78	44	*24*	57	71	*23*	50	66	*20*	37			
DP 2038 B3XF	62	*15*	59	14	62	*37*	80	43	*22*	52	77	*35*	76	71	*32*	59	57	*13*	25
DG 3519 B3XF	51	*5*	61	15	53	*18*	39	48	*35*	83									
DP 2115 B3XF	62	*16*	63	16	63	*39*	85	45	*27*	64	71	*22*	48	70	*30*	56			
Armor 9371 B3XF	63	*17*	64	17	53	*16*	35	47	*32*	76	83	*41*	89	70	*31*	57			
ST 5091 B3XF	64	*18*	74	18	63	*40*	87	47	*31*	74	73	*30*	65	74	*38*	70			
DP 2012 B3XF	68	*19*	82	19	65	*42*	91	52	*39*	93	87	*44*	96	68	*25*	46	69	*43*	83
Frego-bract check	90	*20*	99	20	73	*46*	98	89	*41*	99	98	*45*	99	98	*53*	99	88	*51*	99
Test average					56			45			72			69			63		
LSD 0.10					9			7			9			8			10		

[z]Response to tarnished plant bug was determined by examining white flowers (6 flowers/plot/d for 6 d, replicated 8 times) for presence of anther damage (dirty flower). Accumulate percentage of dirty flowers was determined for each plot.

[y]Percentile (Pctl) determined by (100 * (DF/no. of entries). Rank (rk) of Frego-bract check equals no. of entries each year.

more resistant or equal to the moderately resistant and moderately susceptible check, respectively. These data suggest that a slightly higher number of TPB resistant lines have been found in the cultivar tests than in the Arkansas strain tests. The highest number of resistant cultivars occurred in the 2023 Arkansas Cotton Variety Test, which included 10 entries possessing ThryvOn technology.

The 2023 Arkansas Transgenic Cotton Variety Test included 44 entries (Bourland et al., 2024), 19 of which were included in the 2022 test, 16 in the 2021 test, 13 in the 2020 test, and seven in the 2019 test (Table 8). The 10 entries possessing ThryvOn technology performed well relative to TPB and thus lowered the percent dirty flower ranks of the other entries in 2023. Average percent dirty flowers was much lower in 2022 and 2023 than in the previous 3 yr, which indicated variation in TPB intensity over years. When averaged over years, percent dirty flowers values varied from 43 to 68% among the entries. Three of the 19 entries (DP 2239 B3XF, DG 3528 B3XF, and DG 3519 B3XF) were only tested in the years (2022 and 2023) that had lower average percent dirty flowers. Consequently, the average percent dirty flowers for these cultivars were biased lower compared to cultivars tested in all 5 yr.

To counter this bias, each percent dirty flowers mean was expressed as percentile by dividing percent dirty flowers by number of entries in each test. Two of the three cultivars, which were tested only in 2022 and 2023, had the greatest difference between percent dirty flowers rank and percentile rank. Use of percentile rankings appears to be a good method to compare relatively large number of entries having unbalanced data. Based on percentile rankings, five of the nine entries having the lowest percent dirty flowers were submitted by PhytoGen Seed Company. Over the years, PhytoGen entries have consistently performed well in these TPB tests.

CONCLUSIONS

Using cotton lines with partial resistance to TPB should considerably reduce insecticide use and crop injury. Over the past 21 years, we evaluated cotton lines for response to TPB in small plots using percent dirty flowers as the primary measure of response to TPB. Based on our results above, we conclude: (1) TPB injury as determined by percent dirty flowers was highly dependent on the synchrony of flowering and the observed intensity of TPB populations. Variation in planting dates was not highly effective in obtaining proper synchrony, but late planting dates should be avoided. (2) Both measurements of TPB response (percent dirty flowers and percent darkened anthers) produced comparable data, but percent dirty flowers measurement was preferred because it required less time and was less subjective than estimating percent darkened anthers. (3) Visually rating the boll loads of lines in TPB tests can differentiate variation in TPB response more effectively than percent dirty flowers, particularly in years where TPB populations were not well synchronized with flower production. (4) Nectariless and dense pubescence morphological traits confer some degree of TPB resistance, but similar levels can be found in some nectaried, glabrous types suggesting different mechanisms of resistance. The relationships of TPB resistance to lint yield and earliness of maturity as measured by percent open bolls were not strong, but a tendency for later maturing lines to have lower percent dirty flowers was found. (5) Conventional and transgenic lines appeared to respond similarly to TPB. None are highly resistant, but several express useable levels of partial resistance. Although only tested for one year, ThryvOn technology appears to provide improved TPB resistance relative to conventional and other transgenic lines. (6) Data from these small plots have been used to determine responses to TPB of 360 strains developed in the UA Cotton Breeding Program. Ninety of these strains have been released and these data have been used to help characterize the released lines. And (7) entries in Arkansas Cotton Variety tests have been evaluated for percent dirty flowers in 2006 through 2023. Percentile rankings appear to provide a sound method for comparing unbalanced data of cultivars over years.

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