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David R. Walker · James M. Narvel · H. Roger Boerma · John N. All · Wayne A. Parrott

A QTL that enhances and broadens Bt insect resistance in soybean

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Abstract Effective strategies are needed to manage insect resistance to *Bacillus thuringiensis* (Bt) proteins expressed in transgenic crops. To evaluate a multiple resistance gene pyramiding strategy, eight soybean (*Glycine max*) lines possessing factorial combinations of two quantitative trait loci (QTLs) from plant introduction (PI) 229358 and a synthetic Bt *cry1Ac* gene were developed using marker-assisted selection with simple sequence repeat markers. Field studies were conducted in 2000 and 2001 to evaluate resistance to corn earworm (*Helicoverpa zea*) and soybean looper (*Pseudoplusia includens*), and detached leaf bioassays were used to test antibiosis resistance to Bt-resistant and Bt-susceptible strains of tobacco budworm (TBW; *Heliothis virescens*). Based on defoliation in the field and larval weight gain on detached leaves, lines carrying a combination of *cry1Ac* and the PI 229358 allele at a QTL on linkage group M were significantly more resistant to the lepidopteran pests, including the Bt-resistant TBW strain, than were the other lines. This is the first report of a complementary additive effect between a Bt transgene and a plant insect resistance QTL with an uncharacterized mode of action that was introgressed using marker-assisted selection.

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D. R. Walker · J. M. Narvel · H. R. Boerma · W. A. Parrott
Department of Crop and Soil Sciences, University of Georgia,
Athens, GA, 30602, USA

Present address:
J. M. Narvel
Monsanto Company,
Galena, MD, 21635, USA

J. N. All
Department of Entomology, University of Georgia,
Athens, GA, 30602, USA

W. A. Parrott (✉)
102 Center for Applied Genetic Technologies,
111 Riverbend Road,
Athens, GA, 30602-6810, USA
e-mail: wparrott@uga.edu
Tel.: +706-5838124
Fax: +706-5838120

Introduction

Effective resistance management strategies are essential to prolong the usefulness of insect-resistant crops genetically engineered with *Bacillus thuringiensis* (Bt) δ-endotoxin, or insecticidal crystal (*Cry*) protein genes (Gould 1988a, 1988b; McGaughey and Whalon 1992; McGaughey et al. 1998). Several lepidopteran and coleopteran pests have demonstrated the ability to evolve resistance in the laboratory, and field populations of diamondback moth (*Plutella xylostella* [L.]) have developed resistance to Bt-based pesticides (Tabashnik 1994; Frutos et al. 1999). Current insect resistance management strategies are based on initial resistance gene frequencies and inheritance of resistance in a small number of pest populations and species (McGaughey and Whalon 1992; Caprio 1994; Roush 1996). The "high dose/refugia" strategy mandated by the United States Environmental Protection Agency requires that Bt cotton cultivars and maize hybrids produce at least 25 times the amount of Cry protein needed to kill susceptible individuals to ensure that ≥95% of the insects heterozygous for the resistance allele will also be killed. In addition, a structured refuge composed of non-Bt host plants must be planted to increase opportunities for Bt-resistant insects to mate with Bt-sensitive insects (Roush 1996, 1997; US Environmental Protection Agency 2000). A high dose level of Bt expression may be difficult to achieve for pest species and strains that have an intrinsic tolerance to Cry toxins, such as the cotton bollworm/corn earworm (CEW; *Helicoverpa zea* [Boddie]) and the soybean looper (SBL; *Pseudoplusia includens* [Walker]) (MacIntosh et al. 1990; Mascarenhas and Boethel 1997; Ashfaq et al. 2001; Gore et al. 2001). Supplementary or alternative insect resistance management strategies are therefore needed to mitigate insect resistance to Bt (Gould 1998b).

One strategy would be to pyramid either multiple Bt genes, Bt and unrelated transgenes, or Bt and native plant genes. Expression of multiple Bt genes should increase mortality of insects that have evolved resistance to one of the Bt toxins (Roush 1996; Zhao et al. 2003), and cotton (*Gossypium hirsutum* L.) cultivars carrying *cry2Ab* and *cry1Ac* have been developed (Adamczyk et al. 2001). Native insect resistance in the host plant can increase insect sensitivity to Bt toxins, as demonstrated by reduced survival of lepidopteran larvae on insect-resistant plants treated with Bt (Meade and Hare 1994, 1995; Giustolin et al. 2001). Bt-induced mortality of CEW larvae was also higher on leaves from the resistant soybean plant introduction PI 227687 than on leaves from the susceptible cultivar Centennial (Bell 1978). In these studies, the combined effects of native resistance and Bt treatment were additive and independent. Cotton isolines with higher terpenoid levels and a *cry1Ab* transgene were more resistant to tobacco budworm (TBW; *Heliothis virescens* [F.]) than were transgenic isolines with lower levels of terpenoids (Sachs et al. 1996). The value of transgene/native gene combinations was less evident in another study in which Cry3A-expressing potato (*Solanum tuberosum* L.) clones from an insect-susceptible line and resistant lines with either leptine glycoalkaloids or glandular trichomes caused similar levels of mortality to Colorado potato beetle larvae (Douches et al. 2001; Coombs et al. 2002).

Little is known about the mechanisms of insect resistance in soybean. Three Japanese plant introductions, PI 171451, PI 227687, and PI 229358, show both antixenosis and antibiosis resistance to several lepidopteran and coleopteran pests (Kilen et al. 1977; Lambert and Kilen 1984). Antixenosis discourages insect colonization and/or feeding, whereas antibiosis adversely affects the physiology and/or life history of a pest (Painter 1951; Kogan and Ortman 1978). Exploitation of PI-derived resistance through conventional breeding approaches has been hindered by quantitative inheritance of resistance and by problems with linkage drag, resulting in lines and cultivars with unsatisfactory yields and/or levels of resistance (Kilen and Lambert 1986; Boethel 1999; Lambert and Tyler 1999; Hammond et al. 2001). Rector et al. (1998, 1999, 2000) mapped antixenosis and antibiosis QTLs in these three PIs. PI 171451 and PI 229358 have a resistance allele(s) at a major QTL (229-M) on molecular linkage group (LG) M (Cregan et al. 1999) that conditions both antixenosis and antibiosis to CEW. An antibiosis QTL (229-G) was discovered on LG G of PI 229358, and all three PIs have an antixenosis allele at a QTL on LG H (229-H). Walker et al. (2002) used simple sequence repeat (SSR) markers to backcross 229-M from PI 229358 into Jack-Bt, a line with a synthetic *cry1Ac* gene (Stewart et al. 1996). Jack-Bt shows resistance to CEW, SBL, velvetbean caterpillar (*Anticarsia gemmatalis* [Hübner]), and lesser cornstalk borer (*Elasmopalpus lignosellus* [Zeller]) in the field (Walker et al. 2000). In detached leaf assays, a BC_2F_3 line with the *cry1Ac* + 229-M combination was more resistant to SBL than were related lines carrying either gene alone, but the higher toxicity of Cry1Ac to CEW prevented detection of any effect from 229-M in the transgenic lines (Walker et al. 2002). The current research was conducted to evaluate factorial combinations of a *cry1Ac* transgene and two native soybean insect resistance QTLs, 229-H and 229-M. Resistance was evaluated with CEW and SBL in the field, and with Bt-resistant and sensitive strains of TBW in the laboratory.

Materials and methods

A set of soybean BC_2F_3-derived lines representing all possible combinations of Bt *cry1Ac*, 229-H, and 229-M was developed using marker-assisted selection (MAS). These lines originated from the same Jack-Bt[3] × PI 229358 cross as the BC_2F_3 plants tested by Walker et al. (2002).

DNA extraction, PCR, and electrophoresis protocols for the SSRs were as described by Li et al. (2002). A 10 μL reaction mixture contained 80 ng of template DNA, 1 × PCR buffer (Promega, Madison, Wis., USA), 2.5 mM $MgCl_2$, 100 μM of each dNTP, 0.2 μM each of forward and reverse primers, and 0.5 U of *Taq* DNA polymerase. Sample DNA was amplified using a 32-cycle program in which each cycle involved 25 s denaturation at 94°C, 25 s annealing at 47°C, and 25 s extension at 68°C. This program was preceded by 5 min denaturation at 94°C, and was followed by a 3 min final extension step at 68°C. One of the primers for each marker was labeled with either 6-FAM, HEX, or NED fluorescent tags to allow detection with an ABI Prism 377 semi-automated DNA sequencer (PE-ABI, Foster City, Calif., USA).

One hundred and thirty-one BC_2F_3 plants were genotyped at three SSR loci on LG H (Sat122, Satt442, and Satt541), and at six on LG M (Satt175, Satt245, Satt435, Satt463, Satt536, and Satt540) which flanked the QTLs. These SSR markers spanned approximately 30 cM on LG H and 22 cM on LG M. The antibiosis QTL on LG G was discovered after the pyramiding scheme had begun, so 229-G was not intentionally introgressed, but to prevent potentially confounding effects from 229-G, all of the lines selected were screened with markers to verify that 229-G was not present. The presence or absence of the *cry1Ac* transgene was determined by PCR amplification with sequence-specific primers (Stewart et al. 1996).

To ensure that transgene expression was similar in the transgenic lines, levels of the Cry1Ac protein were measured in leaves from V6- and V7-stage plants (Fehr et al. 1971) using an enzyme-linked immunosorbent assay (ELISA) kit (Agdia, Elkhart, Ind., USA). Protein was extracted from crushed leaf tissue in a 19:1 (v:w) quantity of extraction buffer and diluted 19:1 with protein extraction buffer a second time. Then 100 μl of the total 200 μl reaction volume were transferred to a microtiter plate, and absorbance was measured at 630 nm.

Field studies

Resistance to CEW and SBL was evaluated among the BC_2F_3-derived lines in 2000 and 2001 with "cage" studies like those described in Walker et al. (2000), but the plot design differed in that a single plot consisted of four six-plant hills of the same line. Each plot was surrounded by border hills of either Jack-Bt or SIR-Bt to intercept larvae emigrating from the plot hills. The experimental design for each cage was a randomized complete block with five replications. The treatment design was a 2×2×2 factorial, with each level consisting of the presence or absence of a resistance allele in the homozygous state. Plots were planted on 26 June 2000 and on 22 June 2001. Cages constructed from lumber and PVC pipe were covered with a 0.9×0.9 mm saran mesh to impede the movement of organisms into or out of the studies. Cage dimensions, row spacing, and supplementary lighting to extend the photoperiod were the same as in Walker et al. (2000). Trickle irrigation was used in 2000 to prevent drought stress.

Corn earworm eggs were obtained from the Insect Biology and Population Management Laboratory (USDA-ARS, Tifton, Ga., USA), and SBL eggs were purchased from the Southern Field Crop Insect Management Laboratory (USDA-ARS, Stoneville, Miss., USA). Starting when the plants were at the V3–V4 stage of development (Fehr et al. 1971), approximately 40–45 neonate larvae were applied to each hill using a mechanical device commonly known as a "bazooka", which allows application of controlled doses (Wiseman et al. 1980). Infestations were repeated twice weekly over a 3-week period both years, with two additional infestations in 2000 to compensate for the effects of heavy rainfall.

Visual estimates of defoliation were made periodically over a 3-week period beginning 7 and 10 days after the initial infestations (DAI) in 2000 and 2001, respectively. Percent defoliation was estimated on each of the four hills in a plot, and the mean of the estimates was used in the statistical analyses. Analysis of variance (ANOVA) was conducted on the data from each study to determine whether defoliation differences among the lines were significant ($P \leq 0.05$), and means comparisons were made using Fisher's protected LSD test (Steel and Torrie 1980). Lines were considered a fixed effect, and replications and years as random effects. An additional ANOVA was performed following an arcsin$(\sqrt{\%})$ transformation if heterogeneity of error variances was present (Steel and Torrie 1980). An analysis of the combined data from 2000 and 2001 for defoliation after 3 weeks (21 DAI in 2000 and 24 DAI in 2001) was also conducted in which the *F*-tests were based on expected mean squares (McIntosh 1983).

Detached leaf bioassays

Antibiosis bioassays to evaluate resistance to two TBW strains were conducted with detached leaves in the laboratory. Larvae from the Cry1Ac-resistant YHD2 strain and the related Cry1Ac-susceptible YDK strain of TBW (Gould et al. 1995) were provided by Dr. Fred Gould of North Carolina State University, Raleigh, N.C., USA. The YHD2 strain has been selected for resistance to Cry1Ac in the laboratory, and exhibits cross-resistance to Cry1Aa and Cry1Ab, as well as moderate resistance to Cry1B, Cry1C, and CryIIA (Gould et al. 1995).

Leaflets were collected from trifoliolate leaves of greenhouse plants from each of the eight lines and placed in Petri plates as described in Walker et al. (2002). All lines in a replication were infested with five larvae, after which the Petri plates were sealed with Parafilm (American National Can, Neenah, Wis., USA). The plates were maintained in a growth chamber for 1 week as described by Walker et al. (2002), except that the original leaflets were replaced with fresh ones after 4 days. At the end of an experiment, larvae were transferred to an empty Petri dish overnight and then killed by freezing.

The feeding bioassays were set up as randomized complete blocks with eight replications (Table 1). The experimental unit was the mean weight of the surviving larvae in a Petri dish. The eight lines were tested for significant differences using ANOVA, with soybean lines and TBW strains considered fixed effects. Lines carrying the *cry1Ac* transgene consistently had lower variance than nontransgenic lines, so a log(Y+1) transformation was used to reduce heterogeneity of error variances (Steel and

Table 1 BC_2F_3-derived lines used in feeding bioassays and their insect resistance gene complements. Nomenclature is after Narvel et al. (2001). *SLIR* Soybean lacking insect resistance. *SIR* soybean with insect resistance

Line	*Cry1Ac* (Bt)	Resistance genes	
		QTL allele from LG H of PI 229358 (229-H)	QTL allele from LG M of PI 229358 (229-M)
SLIR	−	−	−
SIR-H	−	+	−
SIR-M	−	−	+
SIR-HM	−	+	+
SIR-Bt	+	−	−
SIR-BtH	+	+	−
SIR-BtM	+	−	+
SIR-BtHM	+	+	+

Torrie 1980). Larval weight means were later reconverted to a milligram scale by calculating the antilog –1 values.

Results and discussion

Based on Mendelian segregation, approximately 87.5% of the genomes of the eight BC_2F_3-derived lines evaluated in this study (outside of the selected regions of LG H and LG M) should have been inherited from the recurrent parent, Jack-Bt. Thus, the lines should provide an unbiased evaluation of the Bt, 229-H, and 229-M genes for insect resistance in a similar genetic background.

Field tests

The field tests measured the combined effects of antibiosis and antixenosis. CEW and SBL were chosen for the field resistance assays because they are important soybean pests which differ in their sensitivity to the Cry1Ac toxin (Stewart et al. 1996; Walker et al. 2002). Both species are also pests of cotton in the Southeast and Delta regions of the USA, where Cry1Ac-expressing cotton cultivars are already widely planted.

Since all possible gene combinations were tested, the treatment effects were subdivided into seven single degree-of-freedom comparisons (Table 2). ANOVA of the defoliation data collected in the field cages across both years revealed that the 229-M QTL and the Bt resistance gene significantly reduced defoliation by both CEW and SBL. The 229-H QTL did not have an effect on defoliation by either pest. There were no interactions among the two QTLs and the Bt transgene for either insect species, indicating that the combined effect of *cry1Ac* and 229-M is additive (Fig. 1).

All lines carrying the Bt *cry1Ac* transgene were significantly less defoliated by CEW and SBL than were the non-Bt lines (Fig. 1). Mean defoliation of the non-Bt lines by CEW (20.4%) was four times greater than in the Bt *cry1Ac* lines (4.5%), and defoliation of the non-Bt lines by SBL was more than twice that of the Bt lines (19.7 versus 8.1%). These results are similar to those previously observed in detached leaf bioassays with BC_2F_3 plants related to the lines tested here (Walker et al. 2002).

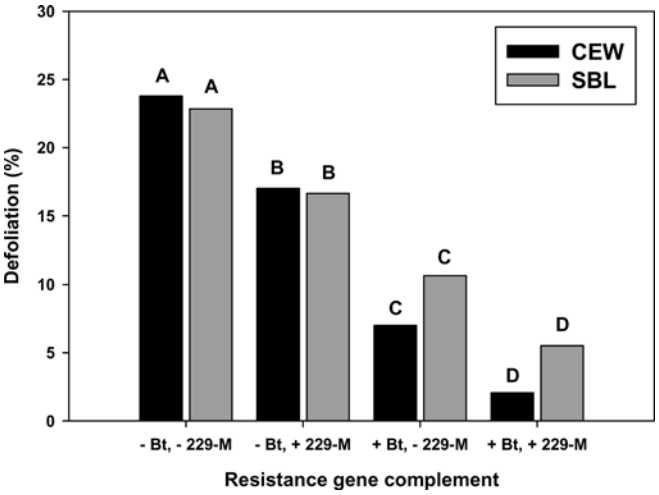

Fig. 1 Mean percent defoliation after 3 weeks by corn earworm (*CEW*) and soybean looper (*SBL*) in field tests in 2000 and 2001 combined over both years. Significant differences between gene complements within an insect species are indicated by different letters ($P \leq 0.05$), based on analysis of transformed data.

The presence of the 229-M QTL also reduced defoliation by CEW and SBL (Fig. 1). Mean defoliation among the lines with 229-M was about one-third lower by both SBL (16.8 vs 11.1%) and CEW (15.4 vs 9.5%) than among lines that did not have 229-M. The combination of *cry1Ac* and 229-M was effective in further reducing defoliation compared with the transgenic lines that did not have 229-M (Fig. 1). The lines with both the *cry1Ac* and 229-M resistance genes averaged 2.0% defoliation by CEW and 5.5% defoliation by SBL, compared with 7.0 and 10.7% defoliation for lines with only *cry1Ac*. Hence the *cry1A* gene was complemented by a native insect resistance gene in soybean, and the combination of the two resulted in a higher level of protection than either provided alone. The Bt + 229-M combination also had reduced CEW larval weights in earlier detached leaf bioassays with BC_2F_3 plants, but the high toxicity of Cry1Ac in those no-

Table 2 Analyses of variance for defoliation by corn earworm (*CEW*) and soybean looper (*SBL*) for data combined over years

Sources of variation	*Df*	CEW		SBL	
		Mean squares	*F*	Mean squares	*F*
Years	1	325.6	24.4**	291.8	6.9*
Replications(years)	8	13.3	1.4	42.5	3.6**
Lines	7	829.7	23.4**	498.8	4.9*
H	(1)	0.2	0.0	8.1	0.1
M	(1)	832.0	23.3**	643.0	6.3*
H × M	(1)	82.8	2.3	21.6	0.2
Bt	(1)	4,829.8	135.1**	2,716.8	26.5**
H × Bt	(1)	19.4	0.5	8.8	0.1
M × Bt	(1)	38.4	1.1	5.6	0.1
H × M × Bt	(1)	5.2	0.1	87.4	0.9
Years × Lines	7	35.5	3.8**	102.5	8.6**
Experimental error	56	9.4		11.9	

*$P \leq 0.05$; **$P \leq 0.01$

choice assays limited the ability to detect significant effects from the native resistance gene (Walker et al. 2002). In a molecular analysis of 15 soybean breeding lines and cultivars selected phenotypically for insect resistance, at least 13 had PI DNA in the region of 229-M, attesting to the importance of this QTL in resistance to various soybean pests (Narvel et al. 2001). The mode(s) of action of 229-M is not yet known, but work currently underway to fine-map and eventually clone this QTL should provide some clues. The results from the present field experiments suggest that *cry1Ac* and 229-M pyramids in soybean lines could be of value in a strategy to manage insect resistance to Bt.

In contrast, the 229-H QTL did not reduce defoliation by either pest (Table 2). This was unexpected, since the QTL on LG H had been detected in mapping populations derived from three different insect-resistant PIs crossed to Cobb. One possible explanation for the lack of an effect in the BC_2F_3-derived lines is that the Jack-Bt allele at this QTL may also condition resistance. Although nontransgenic Jack is more susceptible to lepidopteran pests than PI 229358, it may carry resistance alleles at certain loci, as does the "susceptible" cultivar Cobb (Rector et al. 1999). Another possibility is that while the effects of 229-H appear to be primarily additive (Rector et al. 1998), there may be epistatic interaction(s) involving loci other than 229-M that makes 229-H less effective in a predominantly Jack-Bt genetic background than it is in PI 229358.

Detached leaf bioassays

The assays with the two strains of TBW were designed to evaluate the effects of Bt transgene plus native gene pyramids on a lepidopteran strain that has evolved resistance to the Cry1Ac toxin. Although TBW is not a major pest of soybean, it will readily feed on soybean foliage, and was used here because of the availability of genetically similar Bt-tolerant and Bt-sensitive strains. ANOVA of the combined data for the two TBW strains revealed a significant interaction between the TBW strains and the eight soybean lines ($P<0.01$), so results from the analysis of each strain are presented separately. Most of the observed variance in larval weights was explained by the presence or absence of Bt, 229-M, or both (Table 3). Mean larval weights for the two strains averaged across all lines with the four possible combinations of these two genes are shown in Fig. 2. The 229-H QTL did not reduce TBW larval weights of either strain, but this was expected in these no-choice assays since 229-H is an antixenosis QTL (Table 3).

Both 229-M and the Bt transgene reduced larval weights in the wild-type YDK strain (Table 3). There was also a significant Bt × 229-M interaction, with the effect of 229-M on YDK larval weights only observed in the transgenic lines (Fig. 2). Mean larval weight of YDK larvae in the absence of Cry1Ac was similar in lines with or without 229-M. Among the Bt lines, expression of the transgene in the absence of 229-M resulted in a small but significant reduction in larval weights. This was surprising, since the YDK line is considered to be sensitive to Cry1Ac. The combination of 229-M and Bt, however, reduced larval mean weight to only about 5% of the weight of larvae that had fed on leaves from the lines lacking both genes. This resulted in the Bt × 229-M

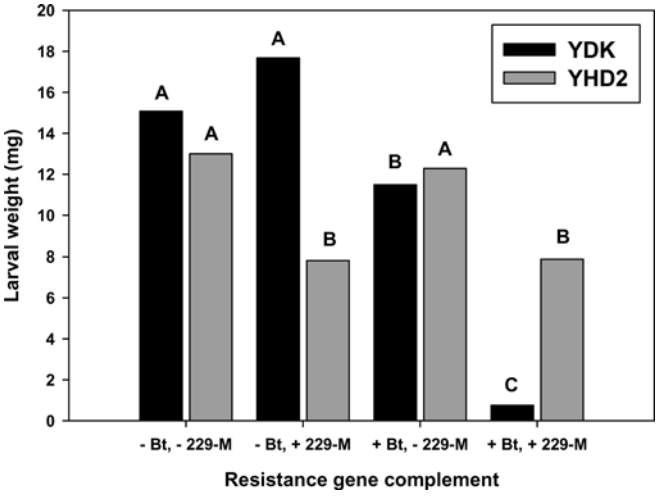

Fig. 2 Larval weights for the Cry1A-susceptible YDK strain and the Cry1Ac-resistant YHD2 strain of tobacco budworm (*TBW*) after feeding for 1 week on detached leaves. Significant differences between gene complements within a TBW strain are indicated by different letters ($P\leq0.05$), based on analysis of transformed data

Table 3 Analyses of variance for larval weights in tobacco budworm strains YDK (Cry1Ac-sensitive) and YHD2 (Cry1Ac-resistant)

Sources of variation	*Df*	YDK		YHD2	
		Mean squares	*F*	Mean squares	*F*
Replications	7	0.27	2.35*	0.28	6.69**
Lines	7	0.91	8.01**	0.23	5.35**
H	(1)	0.17	1.49	0.01	0.18
M	(1)	1.04	9.16**	0.76	17.89**
H × M	(1)	0.46	4.02	0.08	1.92
Bt	(1)	7.15	62.98**	0.16	3.71
H × Bt	(1)	0.04	0.34	0.14	3.31
M × Bt	(1)	1.70	15.00**	0.01	0.29
H × M × Bt	(1)	0.01	0.11	0.02	0.35
Experimental error	48	0.11		0.04	

*$P\leq0.05$; **$P\leq0.01$

interaction observed, and suggests a possible synergistic antibiosis effect on YDK larvae.

For the YHD2 strain, the Bt transgene alone did not reduce larval weights (Table 3, Fig. 2). This was expected, since this TBW strain has been selected for resistance to the Cry1Ac toxin. In contrast to the results seen with YDK, 229-M reduced larval weights by 35–40% in both the transgenic and non-transgenic lines, and the mean larval weights from the lines with 229-M were nearly the same in both transgenic and nontransgenic lines. There was no interaction with Bt, as had been observed with the YDK strain. Bt resistance in the YHD2 strain has been attributed to retrotransposon-mediated disruption of a gene encoding a Cry1Ac-binding, cadherin-like protein (Gahan et al. 2001). The resistance allele is recessive because heterozygous larvae produce enough of the normal form of the protein to bind toxic levels of Cry1Ac.

Gene pyramids for resistance management

It is likely that current high dose/refugia strategy will gradually be supplemented with resistance gene pyramids, since these two strategies are compatible. Compared to deployment of single Bt genes, gene pyramids with two or more Bt transgenes can increase plant protection against certain pests, and may be more effective in delaying the evolution of Bt-resistant pest populations (Gore et al. 2001). Although Monsanto is pursuing a strategy of stacking two Bt genes, there is evidence that pyramiding multiple transgenes may not be equally effective against all targeted pests. In comparison with an isoline expressing only Cry1Ac, a line expressing Cry1Ab and Cry1Ac reduced SBL and beet armyworm populations, but was less effective against fall armyworms (*Spodoptera frugiperda* [J.E. Smith]) and salt marsh caterpillars (*Estigmene acrea* [Drury]) (Adamczyk et al. 2001). A Cry1Ac, Cry2A, and snowdrop (*Galanthus nivalis*) lectin gene pyramid in rice (*Oryza sativa* L.) controlled pests more effectively than any one of the transgenes alone (Maqbool et al. 2001), but *Arabidopsis thaliana* plants with both *cry1Ac* and cowpea (*Vigna unguiculata*) trypsin inhibitor transgenes proved less resistant to four lepidopteran pests than plants with only the *cry1Ac* gene (Santos et al. 1996). Our studies indicate that the combination of the Bt *cry1Ac* transgene and an antixenosis/antibiosis QTL from soybean PI 229358 exhibits an additive and complementary effect on resistance to three lepidopteran pests. In addition, the results from the TBW studies demonstrate the ability of a native plant resistance gene to adversely impact insects that have already evolved a high level of resistance to a Bt toxin. Pyramiding a Bt transgene with one or more complementary native insect resistance genes may reduce the level of transgene expression necessary to meet the "high dose" requirement of the resistance management strategy. If the reduced weights of Bt-resistant larvae feeding on *cry1Ac* + 229-M plant tissue are associated with reduced fitness, then a gene pyramid of this sort should reduce the rate at which the frequency of the resistance gene would increase in a pest population.

The approach we took to pyramid a Bt transgene with native insect resistance in soybean was similar to that used by Sachs et al. (1996) and Coombs et al. (2002) to pyramid resistance traits in cotton and potato, respectively. Our approach differed, however, in that we were working with resistance QTLs identified only with molecular markers, rather than with specific traits or compounds known to be associated with resistance. In addition, we tested our pyramid lines for resistance to a lepidopteran strain that is highly resistant to the Cry1Ac toxin, whereas the strain of TBW which Sachs et al. (1996) used was only moderately tolerant to Cry1Ab.

While stacking multiple Bt genes remains an option, pyramiding a Bt gene with native resistance genes or QTLs may be an effective alternative. Furthermore, the use of one resistance gene combination does not preclude the use of others as supplements to the high dose/refugia Bt resistance management strategy. To the extent that QTLs for insect resistance are identified, these can be readily deployed through the use of marker-assisted selection, even if their mode of action has not yet been characterized.

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