BREEDING RESISTANT SUGARCANE FOR MANAGING THE STEM BORER *DIATRAEA SACCHARALIS*: PROGRESS AND PROSPECTS FOR LOUISIANA

By

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Abstract

THE STEM borer, *Diatraea saccharalis* (F.), is an important insect pest of sugarcane in Louisiana. Growing resistant varieties is a component of the Integrated Pest Management Program as practised in Louisiana for managing this insect; however, the release of stem borer resistant varieties has been intermittent. In 1986, researchers at the USDA, Agricultural Research Service (ARS) and LSU Agricultural Center – the two breeding programs in Louisiana – initiated an effort to increase stem borer resistance without encumbering the Louisiana sugarcane variety program (LSVP) with an additional selection trait. In this approach, clones with high levels of stem borer resistance are developed at the USDA, ARS in Houma via a recurrent selection program for borer resistance (RSB) and these resistant clones are used as parents in crossing. Advancement data from the Louisiana State University AgCenter's sugarcane breeding program were evaluated to determine our success in incorporating the new resistant germplasm into the progeny advancing through this program. A statistical test using the cumulative logit model showed non-significant difference ($P > 0.05$) in advancement rates of clones between the RSB and non-RSB families. However, because of fewer seedlings derived from crosses with at least one RSB parent, very few clones were given permanent variety assignments from the RSB population; only seven in the 1991 to 2002 series. A simulation study was done to determine the effect of increasing selection rates on recovery of RSB clones at the different stages of the breeding program. The cumulative logit model showed that increasing selection rates from Stage I to Stage II will result in a significant increase in the number of RSB clones assigned a variety designation. From this study we propose that the number of resistant crosses be increased and the selection rate of RSB crosses at Stage I be doubled to 13%.

Introduction

Host plant resistance is an important component in the integrated pest management (IPM) of insect pests. Host plant resistance costs the grower little and is compatible with other IPM control tactics (Adkisson and Dyck, 1980). However, unlike diseases that are generally managed with resistant varieties, insect pests of sugarcane are frequently managed by biological control or with insecticides. As a result, sugarcane breeding efforts for insect resistance lag behind efforts devoted to breeding for disease resistance. In fact, susceptibility to diseases has been one of the major incentives for setting up plant breeding programs (Walker, 1987).

Breeding for insect resistance in sugarcane, as with many traits, is likely to be hindered by the complexity of the sugarcane genome and the absence of simply inherited traits (Hogarth, 1987). Several studies have been conducted on the quantitative inheritance of disease resistance in sugarcane (Hogarth *et al.*, 1983 and Hogarth *et al.*, 1993), but few have been conducted for insect resistance. Viator and Henderson (1971) found sugarcane borer resistance to be quantitative in nature, but provided no measures of genetic variation, heritability, or potential gain from selection. White *et al.* (2001) reported that narrow-sense heritabilities for sugarcane borer damage ratings and for percent damaged internodes were high and comparable in magnitude, h^2=0.73 and h^2=0.76 respectively, while Milligan *et al.* (2003) reported broad-sense heritabilities for percent damaged internodes ranging from H=0.29 to H=0.62. Viator and Henderson (1971), White *et al.* (2001), and Kimbeng *et al.* (2006) all presented evidence of a strong additive component to the inheritance of sugarcane borer resistance.

Findings from genetic studies indicate that sugarcane borer resistance could be reliably increased by diligently selecting and crossing among the most resistant parents and then focusing selection on progeny within these crosses. Methods required for the screening and evaluation for resistance have also been established. White (1993) and White *et al.* (1996) discussed screening and evaluation procedures as well as a breeding strategy for increasing levels of sugarcane borer resistance in sugarcane parental populations.

A final, yet critical aspect of breeding for sugarcane borer resistance is genotypic correlations. When selecting rigorously for a few characters, it is important to understand the correlated responses on other important traits. Milligan *et al.* (2003) reported low to moderate broad-sense genetic correlations among five stem borer damage measures (bored internodes, pupation success, moth production, and damage rating) and the yield components (sucrose yield, cane yield, sucrose content, stalk number) from an unselected population. All but one of the damage measures were negatively correlated with sugar yields. Fibre was not determined in this study. White *et al.* (2006) found that varieties with pith were significantly less damaged than those without pith. The authors found that varieties with pith also have higher stalk fibre; a trait that can contribute to lower sugar yields and decreased throughput in raw sugar factories.

The above discussion addresses critical aspects for developing a breeding strategy for sugarcane borer resistance. After initiating a recurrent selection program to identify clones with borer resistance and utilising these as parents, we have become increasingly concerned as to the quality of these clones as parents as no progeny from this material have been released to the grower. Here we summarise 22 years of advancement records from the Louisiana University AgCenter's Sugarcane Variety Program where at least one parent of the bi-parental cross involved a sugarcane borer resistant parent. Drawing from these records, our object was to propose a strategy for increasing the number of selections derived from sugarcane borer resistance parents. Doing so increases the chances of a selection being made or a clone being recycled as a parent for crossing that possesses insect resistance and desirable agronomic characters.

Materials and methods

Recurrent Selection Borer Resistance Program (RSB)

A recurrent selection program for developing sugarcane borer resistant parental clones was started at the USDA, Agricultural Research Service (ARS) in 1986. A summary of procedures and results from the first 10 years of recurrent selection can be found in White *et al.* (1996). These authors report 19 parental clones identified as resistant to the sugarcane borer. By 2008, the number of resistant parental clones had increased to 76; of these, 16 (22%) are currently being used as parents in the Louisiana sugarcane variety program (LSVP). This program is a joint effort between the USDA, ARS, the Louisiana State University AgCenter and the American Sugar Cane League of the U.S.A., Inc.

Incorporation of sugarcane borer resistance from the RSB program

The 1989–1990 crossing campaign was our first opportunity to breed with clones derived from the RSB program. The seedlings derived from these crosses were selected and advanced following the procedures used by the USDA, ARS and the LSU AgCenter's breeding programs. Bischoff and Gravois (2004) provide a detailed summary of the LSVP. Advancement data from the Louisiana State University AgCenter's sugarcane breeding program were evaluated to determine our success in incorporating the new resistant germplasm into the progeny advancing through this program.

Statistical methods

Data derived from the advancements of clones for the 1991 to 2002 crossing series were classified into RSB and non-RSB derived crosses. A cross was designated RSB if it had at least one parent (male or female) from the RSB program. The total number of clones advanced from seedlings that survived winter (Stage I) was calculated for the Stage II (first-clonal stage), Stage III (second-clonal stage), Stage IV (variety yield trials/seed-increase stage) and Stage V (assignment of varieties planted in replicated variety trials).

The multinomial cumulative logit model of the logistic procedure of SAS (SAS Institute, 2007) was used to test if the advancement rates were different between the RSB and non-RSB derived populations. The analysis assumed that the stages of selection were ordered with proportional odds (Agresti, 2007). The cumulative logit model is also known as ordered or ordinal logit model (Allison, 2003). The statistical model used was,

$$\text{Logit}\,[P(Y \leq j)] = \alpha_j + \beta x,\ j = 1, 2, ..., J{-}1, \qquad \text{Equation 1}$$

where α_j is the intercept of the jth stage and β is the coefficient describing the effect of x (RSB or non-RSB population) on the advancement rates. The number of seedlings advanced was used to determine the cumulative proportions that were in turn used to compute the odds ratios (Appendix 1). The model in Equation 1 and the SAS code in Appendix 1 were also used for simulating the effect of increasing the selection rate for the RSB population on the chance of increasing genotypes that reached the assignment stage.

Results

The number of seedlings derived from RSB resistant parents was less than 5% of the seedlings derived from non-RSB parents (Table 1). The trends in Table 1 were consistent across all the crossing series from 1991 to 2002 (data not shown). When the number of seedlings advanced was expressed as a percent of seedlings that survived the winter and plotted against stage of the breeding program (Figure 1), the % advancement of clones from RSB and non-RSB parents was similar across stages.

A statistical test using cumulative logit model (Appendix 1) showed non-significant difference ($P > 0.05$) in advancement rates between RSB and non-RSB families (Table 2). However, because of fewer seedlings derived from RSB crosses, very few genotypes were advanced to replicated variety trials from the RSB population; only seven from the 1991 to 2002 series. We therefore explored potential strategies for increasing advancements of genotypes from the RSB population.

Table 1—The number of seedlings or clones in Stages I, II, III, IV and V from the RSB and non-RSB derived populations, 1991–2002.

Population	Stage I	Stage II	Stage III	Stage IV	Stage V
RSB	17661	1163	228	94	7
Non-RSB	376776	24661	5299	2162	300

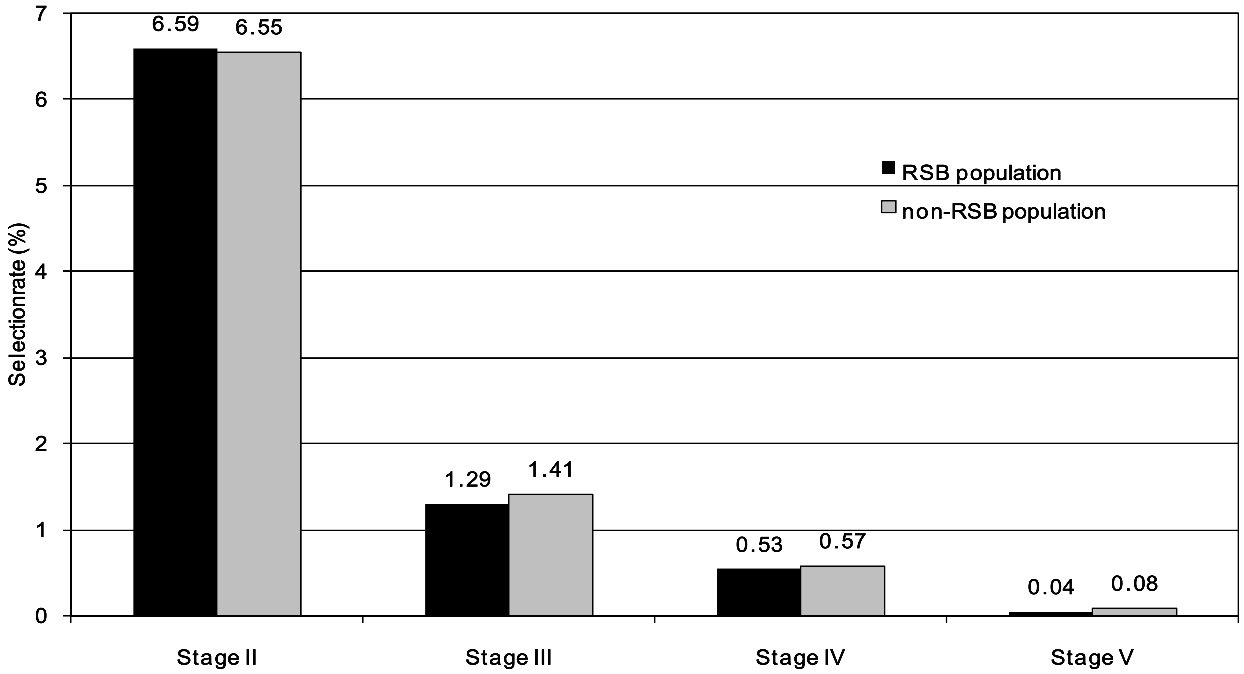

Fig. 1—The selection rates (%) from Stage I to Stages II, III, IV and V from the RSB and non-RSB derived population.

A simulation study was done using the cumulative logit model (Equation 1) and the SAS code (Appendix 1) to determine the effect of increasing selection rates at the different stages of the breeding program. The sensitivity analysis was performed to determine the stages that would produce the greatest effect on the numbers of genotypes reaching Stage V.

Sensitivity analysis enables the variation in the output of a model to be apportioned to the different sources of the variability that occurs in the input variables (Helton *et al*., 2006).

The sensitivity analysis was evaluated by increasing the selection rates between the stages and identifying the stage that would result in the greatest and most significant increase in advancement rates. The selection rate from Stage I to Stage II was the most influential resulting in a significant increase in advancement of RSB derived genotypes (Figure 2).

Increasing advancement rates for Stage IV for the overall breeding population, for example, resulted in marginal increases of advancements to Stage V for genotypes from the RSB population alone. Table 2 and Figure 2 show the impact of increasing selection rates from Stage I to Stage II.

There was a marginal increase in the odds of advancing an RSB genotype compared to the odds of advancing non-RSB from Stage II to Stage III, Stage III to Stage IV, and Stage IV to Stage V.

The simulation study showed that increasing selection rates to 8% (Stage I to Stage II) resulted in a 20% ($P < 0.01$) increase in the odds of advancement to Stage V for genotypes from the RSB population.

A 13% selection rate (double the current selection rate) would almost double the odds of advancement to Stage V and the odds of advancement were tripled at 20% selection rate. The sensitivity analysis also showed that, by adjusting the selection rate at Stage I, the number of genotypes advanced at other stages would significantly ($P < 0.05$) increase (data not shown).

Table 2—The odds ratio of advancing clones from RSB vs non-RSB derived populations, 95 % confidence limits and the Chi-Square tests for different selection rates, Stage I to Stage II.

Selection rate (%)	Odds Ratio (RSB vs non-RSB)	Odds ratio 95 % confidence limits	Wald Chi-Square	Probability > Chi-Square
6.55†	0.98	0.93, 1.04	0.52	0.47
8	1.20	1.14, 1.26	51.21	<0.01
10	1.50	1.43, 1.57	307.29	<0.01
13	1.94	1.86, 2.02	1029.78	<0.01
14	2.09	2.01, 2.17	1348.50	<0.01
17	2.53	2.44, 2.63	2482.34	<0.01
20	2.97	2.87, 3.07	3841.30	<0.01

† Test of the difference in current selection rates between RSB and non-RSB families

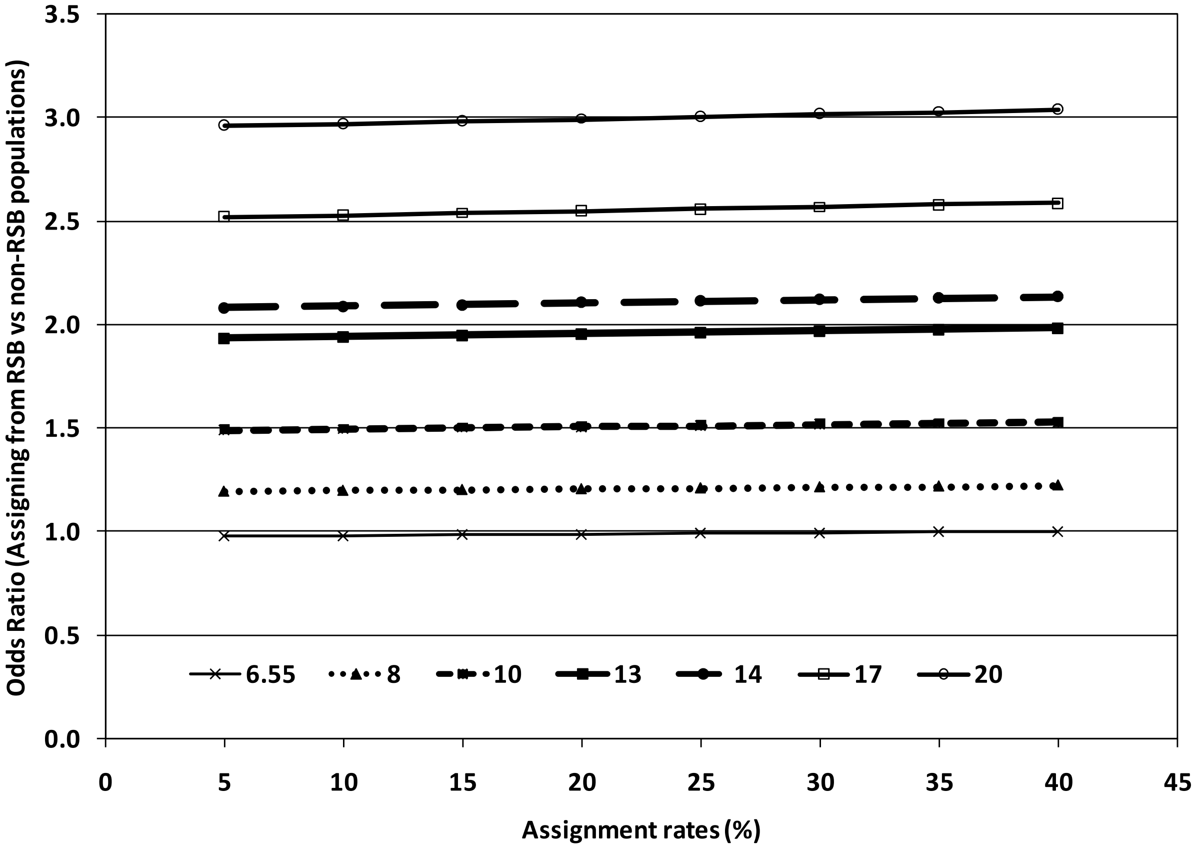

Fig. 2—The odds ratio of assignment for RSB vs non-RSB clones (*y*-axis) plotted against assignment rates (%) (*x*-axis) for different selection rates, Stage I to Stage II.

Discussion

Diatraea saccharalis resistance in sugarcane is a highly heritable trait and, by careful selection of resistant parents, the population mean of progeny evaluated in the LSVP can be shifted to higher levels of resistance. For this strategy to be successful, however, a sufficient representation of these crosses must be planted in initial seedling populations and an appropriate selection rate must be practised. The selection stage that is targeted for increasing the selection rates from the crosses derived from parents resistant to the sugarcane borer is important.

Results from the sensitivity analysis showed that increasing selection rates from Stage I to Stage II would result in a significant increase in the number of genotypes assigned. Increasing the selection rates of the intermediate stages (without increasing selection rates at the seedling stage, Stage I) only resulted in marginal and non-significant increases in assignment rates. Furthermore, increasing the advancement rates from Stage IV alone did not result in significant increases in the number of genotypes advanced to Stage V from RSB families.

The greatest variability in most trait values is in the non-selected seedling population (Stage I). Therefore, increasing selection rates at the seedling stage is expected to capture greater variability and would potentially include those transgressive segregants combining positive agronomic traits with sugarcane borer resistance.

After the seedling stage, further evaluation and selection eliminates those genotypes with poor agronomic performance from a larger pool of sugarcane borer resistant genotypes. Increasing selection rates at later stages did not significantly increase assignment rates because variability is less and many genotypes that combine both high yield potential and borer resistance would have been lost at the seedling selection. Selection in seedlings is inefficient (Cox and Stringer, 1998; Hogarth and Berding, 2006); therefore, higher selection rates from seedlings would provide more genotypes to screen for agronomic traits in later stages.

Consistent strategies are required for increasing the number of borer resistant genotypes that are assigned as well as incorporating borer resistance into the breeding germplasm. Two approaches can be used; selecting more seedlings in Stage I from the RSB parents or increasing the number of crosses made using RSB parents.

The first approach should ensure a greater number of advancements to Stage V, but selecting more seedlings from a smaller population may limit diversity and require the breeder to advance unacceptable clones. Alternatively, increasing the number of crosses made using RSB parentage would result in a larger population to select from and a corresponding increase of RSB genotypes advanced to Stage V at current selection rates. Increasing the number of crosses made with RSB parents would increase the gene pool to select for recombinants that are likely to combine both borer resistance and agronomic traits. The other advantage of increasing RSB parents is that genetic studies can also be carried out to investigate the ability to combine borer resistance and agronomic traits.

Such studies, if positive, would enhance the overall quality of the commercial gene pool, by incorporating an additional desirable trait. Increasing the number of RSB parents does not come without a price. Disadvantages of this approach would be the extra cost (land, labour, equipment, etc.) involved if crosses with these parents add to the number of seedlings evaluated in the program. Alternatively, the number of crosses could remain stable, with a higher percentage of RSB seedlings being planted in the general population. However, this would be done at the expense of traditional commercial breeding crosses.

While the primary objective of commercial breeding is to develop varieties, an additional objective is to improve the gene pool. When selecting from the RSB crosses, in addition to selecting for commercial varieties, we also identify genotypes that may have a small weakness, rendering it not acceptable for commercial production, yet still possessing most of the desired agronomic traits together with borer resistance. Selection of both sets of genotypes will have the positive effect of enhancing the commercial germplasm.

This approach will require evaluating genotype selections targeted to be parental material only, alongside those selected for their commercial potential. The benefits of the RSB program to the commercial program would be enhanced through this approach and thus improve the overall economics of the Louisiana industry.

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Appendix 1. Multinomial cumulative logit SAS code used for data analysis. 1 = RSB population, 0 = non-RSB population; 3, 4, 5, 6, 7 = Stage I, Stage II, Stage III, Stage IV, Stage V, respectively

```
Data one; Input T$ S Count @@; Datalines;
1 3 17661 1 4 1163 1 5 228 1 6 94 1 7    7
0 3 376776 0 4 24661 0 5 5299 0 6 2162 0 7 300;
Proc logistic; weight count; class T; model S=T; run;
```

AMÉLIORATION VARIÉTALE DE LA CANNE À SUCRE POUR LA GESTION DU FOREUR DE LA TIGE *DIATRAEA SACCHARALIS*: PROGRÈS ET PERSPECTIVES POUR LA LOUISIANE

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MOTS CLÉS: Résistance aux Ravageurs, Stratégie d'Amélioration Variétale.

Résumé

LE FOREUR de la tige, *Diatraea saccharalis* (F.), est un ravageur important de la canne à sucre en Louisiane. L'exploitation des variétés résistantes est un élément du programme de lutte intégrée pratiquée en Louisiane pour la gestion de ce ravageur. Toutefois, l'homologation des variétés résistante à été intermittente. En 1986, les chercheurs de l'USDA, l'Agricultural Research Service (ARS) et le LSU Agricultural Center – les deux programmes d'amélioration variétale en Louisiane – ont initié un effort commun pour augmenter la résistance au foreur de la tige sans encombrer le programme variétal de la Louisiane avec un caractère additionnel en sélection. Selon cette approche, les clones avec des niveaux élevés de résistance au foreur de la tige (PSR) développés par l'USDA, ARS à Houma par l'intermédiaire d'un programme de sélection récurrente ont été utilisés comme parents dans le programme d'hybridation. Des données de sélection du programme d'amélioration de la canne à sucre du Louisiana State University AgCenter's ont été évaluées pour déterminer les chances de succès en incorporant le nouveau germoplasme résistant aux progénitures sélectionnées à travers ce programme. Un test statistique, utilisant le modèle cumulatif de logit, a démontré une différence non-significative (P > 0.05) dans les taux d'avancement des clones entre les familles PSR et non-PSR. Cependant, en raison d'un nombre réduit de descendants découlant des croisements avec au moins un parent PSR, très peu de clones de la population du PSR ont été alloués des assignations permanentes au statut de variété; seulement sept dans la série de 1991 à 2002. Une étude de simulation a été entreprise pour déterminer l'effet d'augmenter les taux de sélection sur le recouvrement des clones du PSR à différents stades du programme d'amélioration variétale. Le modèle cumulatif de logit a démontré qu'une augmentation du taux de sélection du Stade I au Stade II pourrait engendrer une augmentation significative des clones du PSR assignés à la désignation de variété. À partir de cette étude, il a été proposé que le nombre de croisements résistants soit augmenté et le taux de sélection des croisements du PSR au Stade I soit doublé à 13%.

MEJORAMIENTO GENÉTICO DE CAÑA DE AZÚCAR PARA EL MANEJO DEL BARREBADOR DEL TALLO DIATRAEA SACCHARALIS: PROGRESOS Y PROYECCIONES PARA LUISIANA

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PALABRAS CLAVE: Resistencia A Insectos, Estrategias de Fitomejoramiento.

Resumen

EL BARRENADOR del tallo, Diatraea saccharalis (F.), es un insecto plaga de la caña de azúcar importante en Luisiana. El uso de variedades resistentes es un componente del Programa de Manejo Integrado de Plagas practicado en Luisiana para este insecto; sin embargo, la entrega de variedades resistentes no ha sido constante. En 1986, investigadores del USDA, Servicio de Investigación Agrícola (ARS) y el Centro de Agricultura de LSU – los dos programas de Luisiana – iniciaron esfuerzos para incrementar la resistencia al barrenador del tallo como un carácter adicional, sin modificar el programa de variedades de Luisiana (LSVP). Con esta proyección, clones con niveles altos de resistencia al barrenador del tallo están siendo desarrollados en USDA, ARS en Houma mediante selección recurrente para resistencia al barrenador (RSB) y estos clones resistentes son usados como parentales para los cruzamientos. Datos avanzados del programa de mejoramiento de caña de Louisiana State University AgCenter's fueron analizados para determinar el éxito de la incorporación del nuevo germoplasma resistente dentro de las progenies avanzadas de todo el programa de selección. El test estadístico usando el modelo logit no mostró diferencias significativas ($P > 0.05$) entre las evaluaciones de clones avanzados entre familias de las RSB y no-RSB. Sin embargo, debido a que pocas plántulas obtenidas de cruzas con al menos una RSB estuvo presente, muy pocos clones fueron asignados códigos varietales desde las poblaciones de RSB, con únicamente siete de las series 1991 a 2002. Se realizó un estudio de simulación para determinar el efecto del incremento de la tasa de selección para la recuperación de clones de las RSB de diferentes estados del programa de cruzas. El modelo logit mostró que incrementando la tasa de selección de Estado I a Estado II incrementará significativo los clones con códigos varietales provenientes del RSB. Con este estudio proponemos que el número de cruzas resistentes se incrementen y la tasa de selección de las cruzas de RSB en Estado I sea duplicado a 13%.