Frequency of Alleles Conferring Resistance to a *Bacillus thuringiensis* Toxin in a Philippine Population of *Scirpophaga incertulas* (Lepidoptera: Pyralidae)

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ABSTRACT Using the F_2 screen methodology, we estimated the frequency of alleles conferring resistance to the Cry1Ab toxin of *Bacillus thuringiensis* Berliner in a Philippine population of the stem borer *Scirpophaga incertulas* (Walker). Evaluation of >450 isofemale lines for survival of F_2 larvae on *cry1Ab* plants did not detect the presence of an allele conferring a high level of resistance. The frequency of such an allele in the sampled population was conservatively estimated to be $<3.6 \times 10^{-3}$ with 95% confidence and a detection probability of 94%. However, there was evidence of the presence of alleles conferring partial resistance to Cry1Ab. The frequency of alleles for partial resistance was estimated as 4.8×10^{-3} with a 95% CI between 1.3×10^{-3} and 1.04×10^{-2} and a detection probability of 94%. Our results suggest that the frequency of alleles conferring resistance to Cry1Ab in the population of *S. incertulas* sampled is not too high to preclude successful implementation of the high dose/refuge resistance management strategy.

KEY WORDS *Bacillus thuringiensis*, *Scirpophaga incertulas*, F_2 screen, Bt rice, Bt resistance

Scirpophaga incertulas (WALKER) (Lepidoptera: Pyralidae) is the most damaging stem borer attacking rice, *Oryza sativa* L., in most parts of tropical Asia (Khan et al. 1991). Chemical control of *S. incertulas* at the reproductive stage of the crop is often ineffective, and only moderate levels of host plant resistance have been identified in rice germplasm. There has therefore been substantial interest in genetic engineering of rice with delta-endotoxin genes from *Bacillus thuringiensis* Berliner (Bt) (e.g., Fujimoto et al. 1993, Cheng et al. 1998, Datta et al. 1998, Maqbool et al. 1998, Alinia et al. 2000b).

Laboratory selection experiments have indicated that the potential to develop resistance to Bt toxins is widespread among insect pest species (Tabashnik 1994). It is generally believed that large scale uniform cultivation of a Bt crop will exert high selection pressure on target pest species and accelerate the buildup of resistance (Gould 1998). An important parameter for studies of resistance risk assessment and the design of resistance management strategies is the initial frequency of alleles conferring resistance to Bt toxins. Indirect estimates of the frequencies of resistance alleles based on laboratory selection experiments placed the figure of 5×10^{-3} to 5×10^{-4} (Tabashnik 1994). A recent study directly estimated the frequency of a major resistance allele in a field population of tobacco budworm, *Heliothis virescens* (F.), to be 1.5×10^{-3} (Gould et al. 1997). Another direct estimate for the diamondback moth, *Plutella xylostella* (L.), found a frequency, in a laboratory-reared susceptible colony, of 0.12 for an allele conferring resistance to at least four Bt toxins (Tabashnik et al. 1997).

Both Gould et al. (1997) and Tabashnik et al. (1997) provided direct estimates by single pair mating of laboratory-selected resistant strains with field-collected insects or a laboratory-reared control strain. Offspring of these crosses, which survive or grow normally on a diagnostic dose of toxin, provide evidence that the field or laboratory insect used in the mating was carrying a resistance allele. This method is very restrictive because it requires that a resistant strain of the pest be available, and it is only able to detect the frequency of resistance alleles found at the same locus as the resistance allele in the resistant colony. An alternative approach to estimating the frequency of resistance alleles, the F_2 screen, has been developed by Andow and Alstad (1998) with modifications by Schneider (1999) and Andow and Alstad (1999). The F_2 screen entails sib-matings within isofemale lines and in principle is able to detect any resistance allele that was initially present in the fertilized eggs of field-collected females. Using the F_2 screen, the frequency of alleles conferring resistance to the Cry1Ab Bt toxin was estimated to be $<5.3 \times 10^{-3}$ and $<2.6 \times 10^{-3}$ in two populations of the European corn borer, *Ostrinia nubilalis* (Hübner) (Andow et al. 1998, 2000).

In this article, we report on the use of the F_2 screen to detect alleles conferring resistance to Cry1Ab in a sample of 450 isofemale lines of *S. incertulas* estab-

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lished from females collected in two adjacent provinces of the Philippines. This research is one component of a program to develop resistance management strategies for Bt rice in Asia (Cohen et al. 1996, Lee et al. 1997, Alinia et al. 2000a).

Materials and Methods

F_2 Screen. As outlined by Andow et al. (1998) the F_2 screen is conducted by (1) sampling mated adult females from natural populations, (2) rearing progeny from each female in isofemale lines and sib-mating the F_1 progeny, (3) collecting eggs from the F_1 parents and screening the F_2 neonates on Bt and control plants, and (4) statistical analysis of the data. By sib-mating the F_1 generation, 1/16 of the F_2 larvae are expected to be homozygous for a resistance allele if such an allele was initially present in the fertilized eggs of a field-collected female. Because each female carries at least four haplotypes [two of her own and two from her mate(s)], each isofemale line enables the characterization of at least four genomes.

Location of Experiments. Insect rearing and production of all plants were done in a greenhouse with no temperature or lighting control. Greenhouse temperatures during the experiments ranged from 23 to 33°C. Exposure of larvae to isolated rice leaves was carried out in an adjoining air-conditioned laboratory maintained at 24–30°C, 70–100% RH, and a natural photoperiod of ≈12:12 (L:D) h.

Plants. For all tests, the Bt rice used was a line of the Iranian variety 'Tarom Molaii' transformed with synthetic *cry1Ab* gene under control of the PEPC promoter (Alinia et al. 2000b). We chose this line because it was known, at vegetative stage, to have stable expression of a sufficient level of toxin. Plants were raised in plastic pots (13 by 15 cm) with sterilized soil. A basal dose of fertilizer to supply 0.52 g N as $(NH_4)_2SO_4$, 0.08 g P as P_2O_5 and 0.25 g K as K_2O per pot was applied. During the dry season (January–May) the N was increased to 0.84 g because of greater solar radiation and higher fertilizer absorption. All of the plants used were at the vegetative growth stage. The control Tarom Molaii plants were derived from the same transformation event as the *cry1Ab* plants, but these plants had lost the *cry1Ab* gene through segregation.

Moth Collection. We conducted two sets of field collections of *S. incertulas* adults. The first set was collected between 24 June and 8 July 1997 from Laguna Province, Philippines, within 10 km of the International Rice Research Institute (IRRI), Los Baños, Laguna. The second set was collected between 27 January and 11 March 1998 from Batangas Province, Philippines, 10–60 km south of IRRI and 0–70 km from the sites where the first set of females was collected. Moths were collected between 0500 and 0800 hours as they rested in rice fields or surrounding weedy vegetation. The field-collected moths were transported to the greenhouse and one male and one female were caged together on a single 30-d-old rice plant, variety 'IR62'. Most field-collected *S. incertulas* females are already mated (J.S.B. and A.M.R., unpublished data) and *S. incertulas* females generally mate only once (Rothschild 1971). The purpose of caging females with males was to enable mating of the few females that might not have already mated in the field. Fertile females produced one to two egg masses within the first 3 d after caging.

Rearing F_1 Larvae and Sib-mating F_1 Adults. Leaf pieces with egg masses were cut from the plants 6–7 d after the caging of the field-collected females and placed in scintillation vials with moist filter paper, eggs of one female per vial. Neonate larvae were used to infest 80-d-old IR62 plants within 12 h of hatching. Larvae hatching from egg masses laid by one female were distributed on 4–8 plants (5–25 larvae per plant), which were placed in Mylar cages (75 by 75 by 100 cm) with a nylon mesh top and side windows. Thus, the progeny of each female parent was reared separately from those of other females. Emerging F_1 adults from each cage were collected daily. Each F_1 female was caged with one male sibling for mating. These F_1 pairs were designated by letters appended to the name of the field-collected parent. For example, P133A through P133J were the 10 F_1 families produced by the sib-mated pairs descended from parent P133.

Testing F_2 Larvae. Egg masses produced by the sib-mated F_1 females were collected from rice plants and placed in scintillation vials as described for the F_1 egg masses. A single leaf with a portion of the leaf sheath of either Bt or non-Bt rice was wrapped in moist cotton, placed in a 9-cm petri dish lined with moist filter paper, and inoculated with 10 neonate F_2 larvae from a single F_1 pair. The petri dish was sealed with masking tape. Larval mortality was recorded after 72 h because several initial tests found that exposure of neonate larvae for 72 h to the leaves and leaf sheaths of the *cry1Ab* plants resulted in 80–93% mortality. To account for possible variation in toxin levels among the test plants, both the insects and test plants were blocked into groups of 10. Within each block, F_2 larvae from each sib-mated F_1 female were exposed to leaves from all 10 plants. One control (non-Bt) plant was also included per block.

To account for the high level of larval *S. incertulas* mortality that occurs even on relatively susceptible plants (Demayo et al. 1994, Alinia et al. 2000b), and the occasional high level of among-family variation in larval survival (Table 1; Demayo et al. 1994), we tested at least 10 F_2 larvae from each of the F_1 families on control plants and calculated a separate value of average control mortality for each of the isofemale lines.

For F_1 families established from the first set of field collections (June and July 1997), larvae surviving after 72 h were gently transferred individually, through a slit made with a scalpel, into an internode of a mature tiller of a potted nontransgenic IR62 plant, to complete larval development. For F_1 families established from the second set of field collections (January–March 1998), surviving larvae were transferred to a fresh excised leaf of a different *cry1Ab* plant for another 72 h (for a total of 144 h of exposure to *cry1Ab* plants). Surviving larvae, if any, were transferred as described

Table 1. Survival on *cry1Ab* plants of *S. incertulas* larvae from the F_2 screen and from a nonselected screenhouse colony

Parental line(s)	F_2 pair	Larvae from F_2 screen			Larvae from screenhouse colony		*t*-test		
		Generation	n^a	Survival (%) on *cry1Ab* plants	n	Survival (%) on *cry1Ab* plants	t	df	P
P133J	SGP29	F_2	6	18.3 ± 4.8		ND[b]			
		F_3	15	28.0 ± 5.4	15	0 ± 0	5.9	28	<0.01[c]
		F_4		ND		ND			
		F_5	94	7.6 ± 1.4	30	9.3 ± 1.4	0.9	122	0.34
P137G, P115A	SGP8	F_2 (P137G)	4	15.0 ± 11.9		ND			
		F_2 (P115A)	3	16.7 ± 3.3		ND			
		F_3 (1st batch)	5	7.7 ± 4.9	5	8.0 ± 5.8	0.0	8	0.99
		F_3 (2nd batch)	9	52.5 ± 0.7	9	18.8 ± 5.4	2.7	16	0.02
		F_4	57	3.8 ± 1.8	13	0 ± 0	2.3	56	0.03[c]

[a] No. replicates; 8–15 larvae per replicate.
[b] Not determined.
[c] *t*-test for means with unequal variance.

to an IR62 plant to complete development. Growth of larvae after each tested was visually noted and recorded.

Testing F_3–F_5 Larvae. In the first set of F_1 families, several of the surviving F_2 larvae transferred to IR62 completed development and emerged as adults. These were sib-mated, or paired across families when sib-mating was not feasible. F_3 larvae were again tested on *cry1Ab* plants for 72 h, along with control larvae originating from a screenhouse colony of *S. incertulas* (Sunio et al. 1997). In two lines, F_4 or F_5 larvae were also tested.

A line with partial resistance was defined as one in which F_3 survival on Bt rice was significantly greater than survival of susceptible control larvae on Bt rice. In addition, for the second set of families, a line with higher survival from the 144-h exposure to Bt rice than susceptible lines was also considered to have partial resistance. The frequency of alleles for partial resistance may be underestimated because many lines could not be tested in the F_3 generation.

A line with major resistance to Bt rice was defined as one that had survival rates on Bt rice similar to the survival rates of susceptible insects on non-Bt rice. In our bioassays with neonate larvae placed on a single leaf sheath and blade in a cup, survival of susceptible insects after 72 h on non-Bt rice was typically ≈50%. This definition would underestimate the frequency of major resistance if resistant isofemale lines did not attain 50% survival rates as a result of inbreeding depression. The frequency of major resistance was further underestimated because many lines could not be tested in the F_3 generation.

Analysis. Data were pooled over both sets of families and analyzed using equation 1 from Andow and Alstad (1998) to estimate the expected allele frequency. Data can be pooled by assuming an uninformative beta prior distribution with $u = v = 1$ (Andow et al. 1999). The 95% credibility intervals were estimated from equation 5 for major resistance and from equation 7 for partial resistance from Andow and Alstad (1999). We calculated these intervals using a Mathematica 4 (Wolfram 1999) program as described in Andow et al. (1999).

We then modified the computer program of Andow and Alstad (1998) to compute detection probabilities. The original program used a uniform value of μ, natural mortality of F_2 larvae for all isofemale lines, and could calculate results only when the average number of F_2 larvae per F_1 female, J, was <60. The modified program uses an experimentally determined value of control mortality (μ) for each line. The program now numerically estimates the detection probability for $J \leq 130$ with rounding errors of $<10^{-9}$. For $J > 130$, if $\mu > 0.54$, then the program simulates the process at least $J \times 10^6$ times to estimate the probability by MonteCarlo simulation, which produces errors of $\approx 10^{-6}$. When $\mu \leq 0.54$, the program defaults to an error of 10^{-7}. The program is available from the authors.

Survival of F_3–F_5 larvae and control larvae from a screenhouse colony were compared by a *t*-test procedure for equal or unequal variances, as appropriate (SAS Institute 1998).

Results

First Set of F_1 Families. Of the 250 females collected, 213 produced enough fertile adults to enable production of sib-mated F_1 families. There was a total of 759 F_1 families, and an average of 3.5 F_1 families produced per isofemale line. Thirty-seven field-collected females produced one F_1 family, 43 produced two families, and 95 produced four or more families. Only four F_1 families had <10 F_2 larvae tested on *cry1Ab* plants, and on average, 37 F_2 larvae per F_1 family were tested. Mortality on control plants was 40.0 ± 1.0%, whereas on test plants it was 95.4 ± 0.3%. Of the 29,359 F_2 larvae from 759 F_1 families tested, 1,337 larvae from 291 F_1 families survived the 72-h exposure on *cry1Ab* plants. Survival was 10% or less in progeny of 181 (62.2%) F_1 families and >25% in progeny of 40 (13.7%) F_1 families.

After transferring the 1,337 surviving F_2 larvae to IR62 plants to complete development, only 44 female and 48 male F_2 adults were obtained. This low level of survival to the adult stage was probably caused by the larvae being weakened after the exposure to Cry1Ab

in addition to the usual level of mortality (≈60%) that occurs during larval development of *S. incertulas*, even on relatively susceptible plants. Five females were sib-mated to obtain F_3 eggs, and the remaining females were mated to males from other isofemale lines because no siblings were available. The five sib-mated pairs came originally from parents P239A (two pairs), P98A, P104E, and P133J. Only one of the F_2 sib-mated second-generation pairs (SGP29) laid eggs and provided F_3 larvae for testing (Table 1). Survival of F_3 larvae of the SGP29 pair after a 72-h exposure to *cry1Ab* plants was significantly higher than that of control larvae from a screenhouse colony. The 134 F_4 larvae of this line were not subjected to selection but were instead reared on IR62 plants to provide a greater number of F_4 adults. When the 870 F_5 larvae of this line were exposed to *cry1Ab* plants, 68 larvae (7.6 ± 1.4%) survived but no F_5 adults were obtained. This survival of the F_5 larvae on *cry1Ab* plants did not differ significantly from that of larvae from the control colony. In addition, 104 F_5 larvae when tested for survival on control plants recorded 44.8 ± 8.4% survival. This survival was comparable to the survival 60.0 ± 1.0% recorded for F_2 larvae on control plants, in tests conducted approximately three months earlier.

F_3 larvae from one cross-family F_2 mating (SGP8) were also tested for survival on *cry1Ab* plants. These tests were done in two batches because of variation in the day of egg hatching. Larval survival of the first batch of F_3 larvae did not differ from that of control larvae, but the second batch of F_3 larvae had significantly higher larval survival than control larvae (Table 1). Survival of the 756 F_4 larvae from the SGP8 line on *cry1Ab* plants was low but still significantly higher than that of control larvae (Table 1). None of the surviving F_4 larvae developed to the adult stage.

If the F_2 larvae that survived selection on *cry1Ab* plants were homozygous for an allele conferring major resistance to Cry1Ab, then the progeny of sib-mated F_2 adults would be expected to have had a survival rate of close to 50% after a 72-h exposure to the *cry1Ab* plants (allowing for 50% "background" mortality attributable to factors other than the effect of the Cry1Ab toxin). Similarly, F_3 larvae produced by cross-family matings of F_2 adults would be expected to have had a survival rate of 50% if both of their F_2 parents were homozygous for the same resistance allele. However, survival after exposure to the *cry1Ab* plants ranged from only 4 to 28% for families of F_3, F_4, and F_5 larvae (Table 1). These relatively low levels of survival for the F_3, F_4, and F_5 larvae suggest that none of the field-collected females were carrying alleles that conferred a high level of resistance to Cry1Ab.

There was a >95% probability of detecting a resistance allele for 16% of the 213 isofemale lines. For <55% of the isofemale lines, the associated probability of detecting a resistance allele was <80%. The experiment-wise detection probability was 71%. To examine how the experiment-wise probability of detecting a resistance allele can change, we analyzed subsets of the data consisting of those isofemale lines, that produced ≥1, 2, 3, 4, and 5 F_1 families (Fig. 1). These data

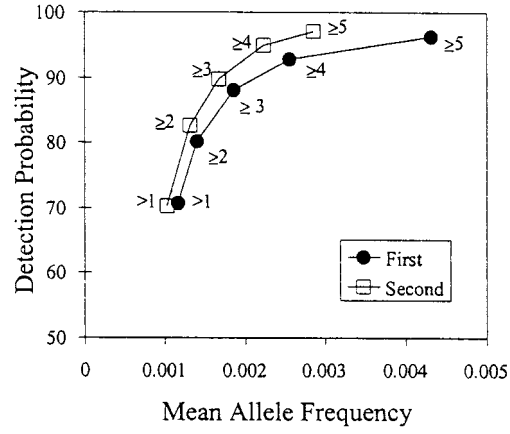

Fig. 1. Trade-off between detection probability and the precision of the estimated resistance allele frequency for the first and second set of families of *S. incertulas*. The numbers by the data points are the number of F_1 families tested per P_1 line, showing that when only data from lines with more F_1 families are analyzed, the detection probability and the estimated allele frequency increase.

show that for data with ≥1 or ≥2 F_1 families per isofemale line, the detection probability is <0.80, implying that a resistance allele would be missed at least 20% of the time, a very high value. For data with ≥4 or ≥5 F_1 families per isofemale line, the detection probability is ≈95%. Consequently, a conservative estimate of the resistance allele frequency would rely only on data from ≥4 F_1 families per isofemale line. A less conservative estimate would be based on ≥3 F_1 families per line, with a detection probability of ≈90%.

Second Set of F_1 Families. Of the ≈1,620 females collected, 241 produced enough fertile adults to enable production of sib-mated F_1 families. There was a total of 1,036 F_1 families, and an average of 4.2 F_1 families produced per isofemale line. Fifty-two field-collected females produced one F_1 family, 40 produced two families, and 110 produced four or more families. None of the F_1 families had <10 F_2 larvae tested on *cry1Ab* plants, and on average, 51.6 F_2 larvae per F_1 family were tested. Mortality on control plants was 60.0 ± 0.2%, whereas on test plants it was 92.2 ± 0.02%. Of the 62,569 F_2 larvae from the 1,036 F_1 families tested, 4,950 larvae survived the 72-h exposure to *cry1Ab* plants. Out of the surviving larvae, 1,415 from 325 F_1 families were retested on *cry1Ab* plants for a second 72-h period. The other surviving larvae were too weak to undergo the second test and were discarded. Only five larvae survived at the end of the second 72-h exposure. Four of these surviving larvae came from one F_1 family: P48A. None of these larvae survived to the adult stage. Based on these results, we concluded that none of the original parents carried a major resistance allele.

Based on larval survival after the first 72-h exposure to *cry1Ab* plants, ≈28% of the lines had a probability of detecting a resistance allele of >95%, and <54% of the lines had a probability of <80%. The experiment-

Table 2. Estimated expected allele frequency (E[*p*]) and 95% CI for resistance and partial resistance in *S. incertulas* to *cry1Ab*-rice

No. F_1 families	No. P_1 lines	Resistance		Partial resistance		Detection probability
		E[*p*]	95% CI	E[*p*]	95% CI	
≥1	454	0.00055	(0,0.00164)	0.0022	(0.0006,0.0048)	70.4
≥2	365	0.00068	(0,0.00204)	0.0027	(0.0007,0.0060)	81.4
≥3	282	0.00088	(0,0.00263)	0.0035	(0.0010,0.0077)	89.0
≥4	206	0.00120	(0,0.00359)	0.0048	(0.0013,0.0104)	94.0
≥5	152	0.00174	(0,0.00485)	0.0048	(0.0010,0.0116)	96.8

The total sample size of P_1 lines with the given number of F_1 families tested per P_1 line is for both sets of families combined. The detection probability is the probability that a resistance or partial resistance allele would be detected given that it were present. A detection probability of 80 implies that one time out of five the resistance allele would not be detected.

wise detection probability was 70%. Again we evaluated how the experiment-wise probability of detecting a resistance allele can change, by analyzing subsets of the data consisting of those isofemale lines, that produced ≥1, 2, 3, 4, and 5 F_1 families (Fig. 1). The second set had slightly higher detection probabilities and lower estimates of allele frequency than the first set (Fig. 1), which shows that experimental methodology had improved somewhat. In general, however, the detection probabilities were similar to those from the first set. Consequently, using the data from isofemale lines with ≥4 F_1 families will provide a conservative estimate of resistance allele frequency.

Major Resistance. Our conservative estimate of allele frequency is based on data with ≥4 F_1 families (Table 2). We observed S = 0 (the number of lines with major resistance), and N = 206 (total number of lines evaluated), so $E[p_R] = 0.00120$ (1.2×10^{-3}) with a 95% CI of (0, 3.6×10^{-3}). In other words, the frequency of resistance is $<3.6 \times 10^{-3}$ with 95% probability. The detection probability associated with this estimate is 94%.

Partial Resistance. Although the survival of F_3, F_4, and F_5 larvae after exposure to *cry1Ab* plants did not approach the level (50%) that would be expected if they were homozygous for alleles that conferred major resistance to Cry1Ab, these larvae did have higher survival than control larvae from an unselected colony (Table 1). This suggests that the selected larvae might have been homozygous for alleles that conferred a partial level of Cry1Ab resistance. If one of the two parents of the SGP29 family and one of the four parents of SGP8 family is considered to have carried an allele for partial resistance, then the number of resistance alleles detected in the first set of 95 families would be 2. In the second set, line P48A contributed four of the five larvae surviving the 144-h screen. These surviving larvae had not yet molted to the second instar but were clearly larger than neonate larvae. Although this line could not be tested in the F_3, it may have had partial resistance. When both sets of lines are pooled, a conservative estimated frequency of alleles conferring partial resistance is $E[p_R] = 0.0048$ (4.8×10^{-3}) with a 95% CI of (1.3×10^{-3}, 1.04×10^{-2}).

Discussion

Our F_2 screens of the two sets of isofemale lines did not detect an allele for major resistance to Cry1Ab. When a subset of the data consisting only of isofemale lines that produced four or more F_1 sib-mated pairs is considered, then the upper 95% CI for the frequency of such a resistance allele in the sampled population is calculated as 3.6×10^{-3} with 94% probability of detection. By comparison, the upper 95% CI of resistance allele frequencies to Cry1Ab in *O. nubilalis* populations from Ames, IA, and LeSueur, MN, were determined as 3.9×10^{-3} and 9.0×10^{-3}, respectively (Andow et al. 1998, 1999; Andow and Alstad 1999). As discussed by these authors, there is a trade-off between detection ability and the precision of the estimate of resistance allele frequency. If we were to consider data from all 454 isofemales lines that we tested, the upper 95% CI of the estimated allele frequency in the *S. incertulas* population sampled would be $<1.6 \times 10^{-3}$ with a detection probability of <70.4%.

If the isofemale lines P48, P133, and either line P137 or P115 are considered to have carried an allele for partial resistance, then the upper 95% CI of the frequency of such an allele in the *S. incertulas* population would be 1.04×10^{-2}. For *O. nubilalis* the upper 95% CI estimates of allele frequencies for partial resistance were 9.4×10^{-3} for Iowa and 1.5×10^{-2} for Minnesota (Andow and Alstad 1999, Andow et al. 1999).

The results of this study indicate that the upper limit of the frequency of alleles conferring major resistance to Cry1Ab in the population of *S. incertulas* sampled approaches the value of 0.001, suggested by Roush (1994) as the maximum value that would enable successful implementation of the high dose/refuge strategy. Screening of 747 total isofemale lines would be required to determine with 95% certainty that the frequency of a major resistance allele in the sampled population is below 0.001 (Andow and Alstad 1999, Schneider 1999).

There are several logistical constraints that should be considered by researchers before deciding to make use of the F_2 screen with *S. incertulas.* As discussed by Andow and Alstad (1998), the F_2 screen is most efficient when the insects can be reared on an artificial diet and the screening can be done in field plots. Field testing of Bt rice has so far taken place only in China (G. Ye, personal communication) and is not likely to

become routine in other countries for several years. Despite extensive effort, an artificial diet that can support complete larval development of *S. incertulas* has not yet been developed (Wang et al. 1983; A. Angeles, M.B.C., and F.G., unpublished data). It is difficult to maintain a colony of *S. incertulas* under optimal rearing conditions, and even more challenging when the larvae are exposed to selection and the isofemale lines are subject to inbreeding depression. The amount of required greenhouse space is a further consideration. We used ≈100 m^2 of greenhouse space to rear the *cry1Ab* and control plants and the large number of F_1 families. This amount of greenhouse space is generally not available to one research group in institutions in countries where *S. incertulas* is a pest, especially in the case of greenhouse space that is approved for the growth of transgenic plants. Of course, the experiment could be conducted in a greenhouse using less space if fewer numbers of isofemale lines were screened at one time.

This study detected evidence of quantitative variation in performance on *cry1Ab* plants within a Philippine population of *S. incertulas.* Alinia et al. (2000a) found a high level of heritability (0.52) for Cry1Ab tolerance within a Philippine population of a second rice stem borer species, *Chilo suppressalis* (Lepidoptera: Crambidae). This quantitative variation could be of significance to the evolution of stem borer resistance to *cry1Ab*-transformed rice cultivars under some conditions. One such condition would be the cultivation of a low-dose cultivar, e.g., the line studied by Alinia et al. (2000b) in which there is a gradual drop in toxin titer after the vegetative stage. Another would be movement of partially resistant larvae between Bt and non-Bt plants in a seed mixture. Larvae of both *S. incertulas* and *C. suppressalis* move between plants during development (Khan et al. 1991, Cohen et al. 1996).

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