Variation in Performance on *cry1Ab*-Transformed and Nontransgenic Rice Varieties Among Populations of *Scirpophaga incertulas* (Lepidoptera: Pyralidae) from Luzon Island, Philippines

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ABSTRACT We quantified variation in performance under greenhouse conditions among seven populations of *Scirpophaga incertulas* (Walker) from Luzon Island, Philippines, on three rice varieties: 'IR58' transformed with the *cry1Ab* gene from *Bacillus thuringiensis* Berliner, and nontransgenic IR58 and IR62. On IR62, *S. incertulas* performance did not differ among provinces for any of the 10 parameters measured, but there was a significant effect of town within province for one parameter, 20-d-old larval weight. Larval survival after 48 h on *cry1Ab*-transformed IR58 did not differ significantly among provinces, but did differ significantly among towns within a province. There was no geographic variation in larval survival after 48 h on control plants of IR58. Surviving insects from the *cry1Ab*-transformed IR58 were transferred to IR62 to complete development. There was no geographic variation in the percentage of insects completing development to adult emergence and the time required by the transferred female insects to complete development. However, there was variation among provinces in male developmental time. The absence of geographic variation on nontransgenic IR58 and the very limited variation on IR62 indicated that there was little variation in general vigor among the *S. incertulas* populations and thus that the variation in performance on *cry1Ab*-transformed IR58 was probably attributable to differences in susceptibility to Cry1Ab.

KEY WORDS *Scirpophaga incertulas, Bacillus thuringiensis,* rice, resistance management

INFORMATION ON AMONG-POPULATION variation in susceptibility to *Bacillus thuringiensis* Berliner toxins can be used to predict the potential for evolution of pest resistance, establish baseline data on susceptibility for use in resistance monitoring programs, and predict geographic variation in the efficacy of transgenic cultivars (Stone and Sims 1993, Tabashnik 1994, van Frankenhuyzsen et al. 1996, Huang et al. 1997, Marçon et al. 1999, Wu et al. 1999). Although rice transformed with *B. thuringiensis* toxins has not yet been released to farmers, *B. thuringiensis* rice is under development in numerous institutions (e.g., Fujimoto et al. 1993, Wünn et al. 1996, Cheng et al. 1998, Maqbool et al. 1998, Alinia et al. 2000b). Consequently, we initiated studies on various aspects of resistance risk assessment and resistance management for two important lepidopterous stem borers of rice in Asia, *Scirpophaga incertulas* (Walker) (Lepidoptera: Pyralidae) and *Chilo suppressalis* (Walker) (Lepidoptera: Crambidae) (Lee et al. 1997, Alinia et al. 2000a, Bentur et al. 2000, Dirie et al. 2000).

Our initial efforts to measure geographic variation of *S. incertulas* responses to *B. thuringiensis* toxins, using artificial diet toxin incorporation bioassays, revealed surprisingly high levels of variation within a 200-km transect of Luzon Island, Philippines (up to 32-fold), and between the Philippines and China (up to 200-fold) (IRRI 1996). However, because this artificial diet bioassay has yielded highly variable LC_{50} estimates for *B. thuringiensis* toxins even within a single *S. incertulas* population (Lee et al. 1997; R. Aguda, M.B.C., and F.G., unpublished data), we suspected that the degree of geographic variation observed was due in part to experimental artifacts. The availability of *cry1Ab*-transformed rice provided us with an alternative method for evaluation of among-population variation in *S. incertulas* susceptibility to the Cry1Ab toxin. In this study, we examined variation in fitness parameters of seven *S. incertulas* populations from three provinces on Luzon, on rice plants transformed with the *cry1Ab* gene (Wünn et al. 1996) and on two moderately susceptible nontransgenic rice varieties.

Materials and Methods

Insect Collection and Rearing. Female moths were collected from farmers' fields in four to five sites within each of five towns in three provinces on Luzon Island, Philippines (Fig. 1), on 28 and 29 August 1996. The collection sites within a town were at least 5 km apart from each other, and towns within a province were separated by ≈25 km. Moths resting on rice plants were collected into a vial and were immediately

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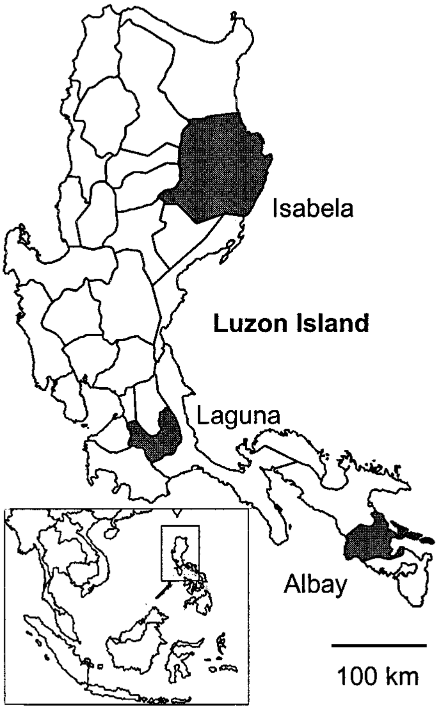

Fig. 1. Location of the three provinces on Luzon Island, Philippines, where *S. incertulas* adults were collected.

transferred to cages containing plants for oviposition. Separate cages were used for collections from each site. Egg masses laid by adults on the leaves and leaf sheaths of the rice plant in each cage were removed and placed individually in sealed and labeled vials 2 d before hatching. Neonate larvae from these eggs were used for all studies.

The number of egg masses obtained from each collection site within a town was generally not sufficient to examine collection site as a unit of geographic variation. We thus pooled egg masses among sites within towns. We were able to obtain a sufficient number of egg masses (>100) for experiments from two towns in Laguna Province (Sta. Maria and Pila) and Isabela Province (Cauayan and Santiago) and three towns in Albay Province (Tiwi, Malinao, and Camalig). Each egg mass (containing ≈100 eggs) was cut in half. Each egg mass probably came from a separate female, because female *S. incertulas* rarely produce more than one large egg mass (J.S.B., unpublished data). The larvae from one-half of each egg mass were distributed among the experimental units for the experiments on varieties 'IR62' and 'IR58' described below. Larvae from the second half of each egg mass were used in artificial diet-based bioassays to estimate LC_{50} values to three *B. thuringiensis* toxins. Unfortunately, these bioassays were ruined by high levels of microbial growth in the artificial diet subsequent to larval infestation.

Variation in Performance on IR62. To compare general vigor among the *S. incertulas* populations, larval survival and weight, pupal weight, adult weight, and developmental time were evaluated on IR62, a moderately susceptible variety. Sixty-day-old IR62 plants grown in 15 by 10-cm clay pots were infested at a rate of 10 larvae per pot. For each town, there were two to five replicate pots for dissection at each of three times: 10, 20, and 27 d after infestation. For each town, an additional 2–15 pots were infested for the purpose of obtaining fitness measurements of adult insects. After infestation, each pot was covered with a cylindrical Mylar cage (1 m tall, with a fine nylon mesh top) to prevent larval movement between pots. Pots were arranged in a randomized complete block design with unequal replication (n = 2–5 replicates per town), with town and dissection day as treatments. Larval and pupal fresh weights were obtained within 2 h of plant dissection, and adult fresh weight within 24 h of emergence.

Variation in Performance on *cry1Ab*-transformed IR58. In these experiments, we used two lines derived from the *cry1Ab* transformation event of IR58 de-

Table 1. Variation among *S. incertulas* populations in larval survival and weight on IR62

Province	Town	Days after infestation							
		Larval survival, %				Larval wt, mg			
		n	10 d[a]	n	20 d[b]	n	10 d[c]	n	20 d[d]
Laguna	St. Maria	2	35.0 ± 15.0	2	35.0 ± 5.0	1	4.0	2	21.0 ± 2.0
	Pila	5	58.0 ± 5.8	5	56.0 ± 8.7	5	4.6 ± 0.4	5	34.8 ± 5.1
Isabela	Cauyan	5	66.0 ± 11.2	5	44.0 ± 11.2	5	4.2 ± 0.9	5	30.5 ± 7.4
	Santiago	5	56.0 ± 13.2	5	46.0 ± 11.2	5	3.6 ± 0.6	5	28.6 ± 3.7
Albay	Tiwi	5	74.0 ± 15.6	5	52.0 ± 10.6	5	5.7 ± 0.9	5	26.3 ± 3.1a
	Malinao	3	26.6 ± 8.8	3	40.0 ± 5.7	3	3.7 ± 0.3	3	51.3 ± 6.3b
	Camalig	5	52.0 ± 12.0	5	54.0 ± 5.1	5	4.8 ± 0.4	5	50.9 ± 2.1b
Laguna		7	51.4 ± 6.7	7	50.0 ± 7.2	6	4.5 ± 0.3	7	30.9 ± 4.4
Isabela		10	61.0 ± 8.4	10	45.0 ± 7.5	10	3.9 ± 0.5	10	29.6 ± 3.9
Albay		13	54.6 ± 8.9	13	50.0 ± 4.6	13	4.9 ± 0.4	13	41.5 ± 3.9

Values are mean ± SE. Means within a column for towns within a province followed by different letters are significantly different ($P < 0.05$, least square means test for unbalanced ANOVA). n, number of replicate pots.

[a] Provinces: $F = 0.6$, df = 2, $P = 0.60$. Towns within provinces: $F = 1.4$, df = 4, $P = 0.26$.

[b] Provinces: $F = 0.3$, df = 2, $P = 0.76$. Towns within provinces: $F = 0.4$, df = 4, $P = 0.81$.

[c] Provinces: $F = 1.5$, df = 2, $P = 0.34$. Towns within provinces: $F = 0.7$, df = 4, $P = 0.62$.

[d] Provinces: $F = 1.2$, df = 2, $P = 0.39$. Towns within provinces: $F = 4.4$, df = 4, $P = 0.01$.

Table 2. Variation among *S. incertulas* populations in pupal fresh weight on IR62

Province	Town	Weight, mg			
		n	Female[a]	*n*	Male[b]
Laguna	St. Maria	2 (2)	42.9 ± 12	1 (3)	35.9
	Pila	1 (4)	58.1	2 (4)	26.9 ± 2.4
Isabela	Cauyan	4 (5)	37.7 ± 5.5	3 (6)	28.5 ± 2.3
	Santiago	4 (9)	51.3 ± 3.3	5 (14)	29.3 ± 0.7
Albay	Tiwi	4 (9)	39.3 ± 6.2	4 (9)	26.6 ± 1.9
	Malinao	2 (5)	36.4 ± 6.8	1 (2)	24.3
	Camalig	4 (10)	46.6 ± 10.1	4 (10)	29.1 ± 2.2
Laguna		3	47.9 ± 8.7	3	29.9 ± 3.3
Isabela		8	44.5 ± 3.9	8	29.0 ± 0.9
Albay		10	41.7 ± 4.7	9	27.5 ± 1.3

Values are mean ± SE. Means for towns within a province and for provinces are not significantly different ($P > 0.05$, least square means test for unbalanced ANOVA). *n*, number of replicate pots, with total number of insects in parentheses.

[a] Provinces: $F = 0.7$, df = 2, $P = 0.54$. Towns within provinces: $F = 1.0$, df = 4, $P = 0.48$.

[b] Provinces: $F = 1.0$, df = 2, $P = 0.45$. Towns within provinces: $F = 0.2$, df = 4, $P = 0.18$.

scribed by Wünn et al. (1996), which contains a codon-optimized, truncated *cry1Ab* gene under control of the CaMV 35S promoter. The plants were grown from the second generation of seed produced after transformation and tissue culture. One line, 73-4, contained the *cry1Ab* gene. The second line, 73-24, had lost the cry1Ab gene through segregation and served as a control. In preliminary experiments, we found that a 96-h exposure of neonate *S. incertulas* to stem pieces of line 73-4 resulted in 93% mortality.

Neonate larvae were exposed in batches of 20-30 to 15- to 20-d-old tillers removed from plants of the *cry1Ab*-transformed and control lines. The roots of the tillers were left intact and were wrapped in moist absorbent cotton. Infested tillers were enclosed in 450-ml plastic cups. The cups were arranged in a completely randomized design. The number of cups (replicates) established for each town varied, depending on the number of available larvae. There were 4–33 cups per town containing tillers from *cry1Ab*-transformed plants, and two to five cups with tillers from control plants. Larval mortality was recorded after 48 h. All surviving larvae from each *cry1Ab*-transformed tiller were carefully transferred to the ripening panicle of a potted IR62 plant, through a slit made into an internode below the panicle, to complete larval development and pupation. Depending on the number of surviving larvae from the *cry1Ab* plants, two to five replicate pots of IR62 were established per town. The pots were arranged in a completely randomized design. The percentage of larvae per pot completing development to adult emergence and the time to adult emergence were recorded.

Statistical Analysis. Percentage data were transformed to arcsine of the square root. Data were analyzed with SAS (SAS Institute 1998) using nested analysis of variance (ANOVA) for unbalanced data, with towns nested within provinces. For tests of significance, the "H =" option was used with town within province as the error term. Least square means were compared among provinces and towns within provinces.

Results

Variation in Performance on IR62. Larval survival and weight after 10 and 20 d on IR62 did not vary significantly among the provinces of Laguna, Isabela, and Albay or among towns within Laguna or Isabela (Table 1). Within Albay Province there was significant variation among towns in the weight of 20-d-old larvae, with larvae from the town of Tiwi weighing significantly less than larvae from Malinao and Camalig. Pupal weight, developmental time, and adult weight did not vary significantly among provinces or among towns within provinces, for either males or females (Tables 2 and 3).

Variation in Performance on *cry1Ab*-Transformed IR58. Larval survival after 48 h on tillers from *cry1Ab*-transformed IR58 did not differ significantly among provinces, but did differ significantly among towns within a province (Table 4). Specifically, within Isa-

Table 3. Variation among *S. incertulas* populations in developmental time and adult fresh weight on IR62

Province	Town	Developmental time, d				Adult wt, mg			
		n	Female[a]	*n*	Male[b]	*n*	Female[c]	*n*	Male[d]
Laguna	St. Maria	2 (2)	37.5 ± 3.5	2 (6)	37.3 ± 0.3	2	24.1 ± 6.8	2	10.0 ± 0.8
	Pila	8 (17)	34.5 ± 0.7	9 (19)	33.4 ± 1.2	8	29.0 ± 1.8	9	10.9 ± 0.5
Isabela	Cauyan	11 (27)	35.2 ± 0.6	15 (27)	34.2 ± 0.8	11	33.0 ± 1.4	15	11.6 ± 0.6
	Santiago	9 (25)	34.7 ± 0.5	12 (25)	33.6 ± 0.7	9	30.9 ± 1.1	12	10.5 ± 0.5
Albay	Tiwi	11 (33)	34.0 ± 0.5	11 (36)	34.5 ± 0.7	11	30.8 ± 1.6	11	11.5 ± 0.3
	Malinao	3 (5)	35.3 ± 0.9	4 (9)	35.0 ± 0.9	3	30.9 ± 6.7	4	11.9 ± 1.2
	Camalig	12 (30)	35.2 ± 0.9	14 (34)	33.1 ± 0.8	12	30.4 ± 1.2	14	10.8 ± 0.7
Laguna		10	35.1 ± 0.9	11	34.1 ± 1.1	10	28.0 ± 1.9	11	10.7 ± 0.5
Isabela		20	35.0 ± 0.4	27	34.0 ± 0.5	20	32.1 ± 0.9	27	11.1 ± 0.4
Albay		26	34.7 ± 0.5	29	33.9 ± 0.5	26	30.7 ± 1.1	29	11.2 ± 0.4

Values are mean ± SE. Means for towns within a province and for provinces are not significantly different ($P > 0.05$, least square means test for unbalanced ANOVA). *n*, number of replicate pots, with total number of insects in parentheses.

[a] Provinces: $F = 0.6$, df = 2, $P = 0.58$. Towns within provinces: $F = 2.3$, df = 4, $P = 0.83$.

[b] Provinces: $F = 0.5$, df = 2, $P = 0.65$. Towns within provinces: $F = 1.1$, df = 4, $P = 0.37$.

[c] Provinces: $F = 2.0$, df = 2, $P = 0.26$. Towns within provinces: $F = 0.2$, df = 4, $P = 0.93$.

[d] Provinces: $F = 0.9$, df = 2, $P = 0.48$. Towns within provinces: $F = 0.3$, df = 4, $P = 0.89$.

Table 4. Variation among *S. incertulas* population in larval survival and adult emergence after 48 h exposure to *cry1Ab*-transformed and control plants of IR58

		Larval survival (%)						Adult emergence (%) after larval transfer from *cry1Ab*-IR58 to IR62[c]
Province	Town	*n*	Control plants[a]	*n*	*cry1Ab*-transformed plants[b]		*n*	
Laguna	St. Maria	4	78.1 ± 2.6	4	25.0 ± 6.5	**	4	6.7 ± 3.3
	Pila	2	71.3 ± 8.8	12	27.7 ± 6.9	*	2	2.5 ± 1.8
Isabela	Cauyan	4	56.3 ± 3.9	33	26.1 ± 2.8a	*	4	0.9 ± 0.6
	Santiago	4	51.9 ± 8.9	25	46.1 ± 4.5b	NS	4	1.1 ± 0.7
Albay	Tiwi	5	49.0 ± 1.0	26	28.4 ± 3.9	*	5	1.8 ± 1.3
	Malinao	3	62.5 ± 11.3	9	44.1 ± 5.6	NS	2	2.2 ± 1.4
	Camalig	3	69.1 ± 13.7	16	32.9 ± 7.0	NS	3	1.9 ± 1.3
Laguna		6	75.8 ± 3.1	16	27.1 ± 5.3	**	6	3.5 ± 1.6
Isabela		8	54.1 ± 4.6	58	34.7 ± 2.8	*	8	1.0 ± 0.5
Albay		11	58.2 ± 5.0	51	32.6 ± 3.1	**	10	1.9 ± 0.8

Values are mean ± SE. Means for towns within a province followed by different letters are significantly different ($P > 0.05$, least square means test for unbalanced ANOVA). *n*, no. of replicate cups (larval survival) or pots (adult emergence). Comparison of larval survival within a row; *t*-test. NS, nonsignificant; * and **, significant at the 5 and 1% levels, respectively. Towns: St. Maria, $t = 7.2$, df = 6, $P < 0.01$; Pila, $t = 2.3$, df = 12, $P = 0.04$; Cauyan, $t = 3.3$, df = 35, $P < 0.01$; Santiago, $t = 0.5$, df = 27, $P = 0.63$; Tiwi, $t = 2.2$, df = 29, $P = 0.03$; Malinao, $t = 1.6$, df = 10, $P = 0.14$; Camalig, $t = 2.0$, df = 17, $P = 0.07$. Provinces: Laguna, $t = 5$, df = 20, $P < 0.01$; Isabela, $t = 2.4$, df = 64, $P = 0.02$; Albay, $t = 3.4$, df = 60, P < 0.01.

[a] Provinces: $F = 2.6$, df = 2, $P = 0.19$. Towns within provinces: $F = 1.3$, df = 4, $P = 0.29$.

[b] Provinces: $F = 0.3$, df = 2, $P = 0.78$. Towns within provinces: $F = 3.8$, df = 4, $P = 0.01$.

[c] Provinces: $F = 5.3$, df = 2, $P = 0.07$. Towns within provinces: $F = 0.3$, df = 4, $P = 0.85$.

bela Province, survival was lower for the town for Cauyan than for Santiago. Larval survival on tillers from control plants did not differ among provinces or among towns within provinces. Within towns, larval survival on control tillers was significantly higher than on *cry1Ab*-transformed tillers in four of the seven towns, as tested by pairwise comparisons. In the remaining three towns, larval survival was numerically higher on control tillers, but not significantly so.

The percentage of insects that completed development to adult emergence, after transfer from *cry1Ab*-transformed tillers of IR58 to potted plants of nontransgenic IR62, was low for all towns, ranging from 0.9 to 6.7% (Table 4). These values did not vary significantly among provinces or among towns within provinces. The mean time to adult emergence varied significantly among provinces for males and was significantly higher for Laguna Province than for Isabela Province (Table 5). Developmental time did not differ among towns within provinces, for either sex.

Table 5. Variation among *S. incertulas* populations in developmental time of adults developing from larvae exposed to *cry1Ab*-transformed IR58

Province	Town	*n*	Female[a]	*n*	Male[b]
Laguna	St. Maria	2 (2)	38.5 ± 1.5	3 (6)	39.3 ± 0.8
	Pila	2 (4)	38.3 ± 1.8	2 (5)	38.3 ± 1.3
Isabela	Cauyan	2 (4)	36.2 ± 3.8	2 (5)	36.8 ± 0.2
	Santiago	2 (2)	32.0 ± 3.0	2 (5)	37.0 ± 0.0
Albay	Tiwi	4 (5)	36.9 ± 0.7	2 (11)	37.4 ± 0.4
	Malinao	1 (1)	37.0	2 (2)	36.5 ± 3.5
	Camalig	2 (3)	36.5 ± 0.5	2 (6)	35.3 ± 0.8
Laguna		4	38.4 ± 0.9	5	38.9 ± 0.6a
Isabela		4	34.1 ± 2.3	4	36.9 ± 0.1b
Albay		7	36.8 ± 0.4	6	36.4 ± 1.0ab

Values are mean ± SE. Means within a column for provinces followed by different letters are significantly different ($P < 0.05$, least square means test for unbalanced ANOVA). *n*, number of replicate pots, with total number of insects in parentheses.

[a] Provinces: $F = 0.5$, df = 2, $P = 0.64$. Towns within provinces: $F = 1.3$, df = 4, $P = 0.41$.

[b] Provinces: $F = 6.5$, df = 2, $P = 0.06$. Towns within provinces: $F = 0.1$, df = 4, $P = 0.98$.

Discussion

Out of the 10 variables observed for insects on IR62, in only one instance (weight of 20-d-old larvae on IR62) was there a significant effect of town within province (Table 1), and in no case was there significant variation among provinces. There were no differences among populations in larval survival after 48 h on tillers of nontransgenic IR58, but variation in larval survival on *cry1Ab*-transformed IR58 was significant among towns within Isabela Province (Table 4). The very limited variation on the nontransgenic varieties indicates that there was little variation in general vigor and in adaptation to the nontransgenic varieties among the *S. incertulas* populations, and suggests that the variation in performance on *cry1Ab*-transformed plants was attributable to differences in susceptibility to Cry1Ab. The low survival of *S. incertulas* on control plants (Table 1) is typical of this species, and is in part attributable to the strong tendency of neonate larvae to disperse from the plant on which they eclose or are placed upon shortly after eclosion (Alinia et al. 2000b, Dirie et al. 2000).

One of the purposes of conducting this study was to obtain an estimate of variation of Cry1Ab susceptibility among *S. incertulas* populations to contrast with that obtained in artificial diet-based bioassays. In the artificial diet assays we found surprisingly high levels of variation (up to 32-fold) in LC_{50} estimates for Cry1Ac, Cry1C, and Cry2A within a 200-km transect of Luzon Island, Philippines (IRRI 1996). Variation among populations was observed in our experiments using *cry1Ab*-transformed plants as well (Tables 4 and 5). Because different biological parameters were measured and different toxins were used, it is not possible to directly compare the magnitude of geographic variation observed in the experiments using artificial diet with that observed in the experiments using transgenic plants. However, the limited geographic variation we observed in the current study indicates that *cry1Ab*-transformed rice will show similar protection against *S. incertulas* throughout Luzon after its initial release. The highest and lowest town means for larval survival after 48 h of exposure to *cry1Ab*-transformed rice differed by only 1.8-fold (Table 4).

Differential exposure to Bt sprays is unlikely to explain the variation in Cry1Ab susceptibility among *S. incertulas* populations on Luzon. Bt sprays are seldom used on rice in the Philippines, and although there are a few reports of wild grasses serving as hosts of *S. incertulas,* rice is its only cultivated host (Dale 1994).

In a greenhouse study of five *S. incertulas* populations collected across a 600-km transect of Luzon Island, Demayo et al. (1994) observed variation in fitness on nine rice hosts (cultivated varieties and wild species). This variation, which was shown to be most likely attributable to genetic differentiation, was not associated with adaptation to locally popular rice varieties. Demayo et al. (1994) predicted that variation in performance on rice transformed with Bt toxin genes would also exist among *S. incertulas* populations, a prediction supported by the results of the current study. Using the "F_2 screen" methodology, we have also observed within-population variation in performance on *cry1Ab*-transformed rice among isofemale lines of *S. incertulas* established from moths collected in Laguna and Batangas Provinces (Bentur et al. 2000). The quantitative variation in performance on *cry1Ab* plants observed in the current study and the F_2 screen indicate the potential for the evolution of Cry1Ab resistance in *S. incertulas* populations on Luzon.

Studies of other lepidopterous species have found variable amounts of geographic variation in LC_{50} estimates for purified *B. thuringiensis* toxins. Marçon et al. (1999) found only a four-fold variation in LC_{50} estimates for Cry1Ab and Cry1Ac among *Ostrinia nubilalis* (Hübner) populations from the United States and Italy. A survey of *Helicoverpa zea* (Boddie) and *Heliothis virescens* (F.) populations across the southern United States and the Virgin Islands found eight-fold and 16-fold variation in LC_{50} estimates for Cry1Ac, respectively (Stone and Sims 1993). LC_{50} estimates for Cry1Ac for populations of *Helicoverpa armigera* (Hübner) from five regions of China were found to vary up to 100-fold (Wu et al. 1999). It is recognized that some variation in LC_{50} estimates within and among populations is attributable to "natural variation" caused by slight differences in environmental factors and genetic background (Robertson et al. 1995). Measurements of insect development and survival on transgenic plants provide information on the biological significance of the variation observed in LC_{50} estimates.

Relative to the enormous geographic area over which *S. incertulas* attacks rice in Asia (from Indonesia in the south and east to Afghanistan in the north and west), and the area over which Bt rice may eventually be grown, the area sampled in this study was extremely small. There is clearly a need for testing many additional populations, to obtain a more complete picture of variation among *S. incertulas* populations in susceptibility to *B. thuringiensis* toxins. We have made extensive efforts to improve the repeatability of LC_{50} estimates for Cry1Ab using toxin incorporation in artificial diet, with only limited success (R. Aguda, M.B.C, and F.G., unpublished data). Unless repeatable LC_{50} estimates can be obtained with such assays, researchers conducting further studies of geographic variation in Cry1Ab susceptibility should consider assays with *cry1Ab*-transformed rice, such as those described in this paper.

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