BEET ARMYWORM RESISTANCE TO CRY1AC

John K. Moulton and Timothy J. Dennehy
Extension Arthropod Resistance Management Laboratory
Department of Entomology
University of Arizona
Tucson, AZ

Abstract

Susceptibility of beet armyworm, *Spodoptera exigua* Hübner, to the Bt toxin, Cry1Ac, expressed in first generation of transgenic cotton plants was evaluated using a laboratory strain and several foreign and United States field strains. A diet-incorporation assay of neonate larvae was used. Susceptibility was estimated by the degree to which Cry1Ac inhibited larval growth from the first to fifth instar. Regression analysis of larval weights against log concentration of Cry1Ac yielded slope and intercept values that were used to compute I_{50}s, defined as the amount of Cry1Ac that resulted in a fifty percent reduction in larval growth. Three populations exhibiting reduced susceptibility to Cry1Ac were selected on diet containing 1000 micrograms of Cry1Ac per gram of diet. I_{50}s for non-selected populations ranged from 0.0477 micrograms Cry1Ac per gram of diet for the laboratory reference strain to 4.31 micrograms of Cry1Ac per gram of diet for a field strain collected from Yuma, Arizona. Selection of a strain from Belle Glade, Florida, with Cry1Ac yielded the lowest susceptibility to this toxin. Prior to selection, the I_{50} was 2.43 micrograms of Cry1Ac per gram of diet; after selection the I_{50} was 17.4 micrograms of Cry1Ac per gram of diet. Thus selection reduced susceptibility of the Belle Glade, Florida strain to Cry1Ac by 7.2-fold and yielded susceptibility that was 360-fold less than the laboratory reference strain. Selection also reduced susceptibility of an Arizona (Dome Valley) population by 3-fold. Our results demonstrate the presence of large (>25-fold) differences in susceptibility of Arizona beet armyworm populations to Cry1Ac. Furthermore, the fact that resistance was elevated three to seven-fold in two selected strains provided evidence of a genetic basis of resistance to Cry1Ac in *S. exigua*.

Introduction

Cotton genetically modified to express an insecticidal protein from *Bacillus thuringiensis* has been widely adopted in Arizona since 1996 (Patin et al. 1999). Overall, Bollguard® cotton (Monsanto Corp.) has been planted on approximately 60% of cotton acreage in Arizona since 1998 but in some areas has comprised as much as 90% of cotton acreage (Sims et al., this volume). Such intensive use of Bt cotton in the U.S. has resulted in considerable attention to the threat of resistance to Bt toxins by the principle target pests, bollworm (*Helicoverpa zea* (Boddie)), budworm (*Heliothis virescens* (Fabricius)), and pink bollworm (*Pectinophora gossypiella* (Saunders)) (Gould and Tabashnik 1998).

A central question in the public debate regarding the benefits and risks of transgenic Bt technology regards the effects that Bt crops may have on farmers who do not use them but rely upon conventional foliar spray applications of Bt to control lepidopteran pests. The Union of Concerned Scientists has called for more work to ensure that transgenic Bt crops will not cause growers that use organic or integrated pest management practices to lose efficacy of foliar sprays of Bt, therein undermining their prospects for success (Mellon and Rissler 1998). Greenpeace, the International Federation of Organic Agricultural Movements, and over seventy other environmental groups stated this concern even more strongly in a lawsuit filed in 1999 against the U.S. EPA. They deduced that the evidence overwhelmingly backs the conclusion that genetically engineered crops are an imminent threat to farmers and the environment (CTA 1999). A 1998 EPA Scientific Advisory Panel agreed that some secondary pests may also be at risk for evolving resistance to Bt toxins because of Bt crops (EPA 1998). The work described in this paper is part of a multi-year study that strives to bring field data to bear on this controversy.

The beet armyworm, *Spodoptera exigua* Hübner, is a widely distributed, highly mobile, and polyphagous pest of cotton and many other crops worldwide (Metcalf et al. 1962). Beet armyworm is suppressed by Bollgard cotton (Adamczyk et al., 1998, Wilson et al. 1992, Henneberry, this volume). and efforts are underway to enhance toxicity of future generations of transgenic Bt cotton to this pest (Sivasupramanian, this volume). In 1999, we began testing susceptibility of collections of beet armyworm to Cry1Ac, with emphasis on Arizona populations. This work will be expanded in the coming two years and susceptibility of populations will be contrasted with site- and region-specific estimates of use of Bt cotton. In this paper we describe large differences in susceptibility to Cry1Ac observed in beet armyworm populations collected from Arizona, Florida, Spain, and Thailand. We then report on magnification of resistance to Cry1Ac in this pest resulting from exposure to Cry1Ac in the laboratory.

Materials and Methods

Cultures

Field populations of *S. exigua* were established from samples collected by members of our laboratory (EARML), Dow Agrosciences, and Rohm and Haas Company from August 1998 to October 2000. Arizona field populations were established from larvae collected from cotton or adults attracted to ultraviolet lights. Field populations from elsewhere were shipped to EARML as surface sterilized eggs under the terms of USDA-APHIS permit number 39094, and the resulting neonates were allowed to complete development by placing them on the aforementioned artificial diet. The susceptible reference strain was established from eggs shipped to EARML from the USDA-Western Cotton Research Laboratory (WCRL) in Phoenix, AZ.

Rearing

All cultures of *Spodoptera exigua* larvae were reared on Stonefly Industry's *Heliothis* Premix diet, to which was added 5 ml of formalin and 1.5 g of aureomycin (chlortetracycline HCL, Fort Dodge Animal Health, Fort Dodge, IA) to each 2000 g of prepared diet (500 g diet, 1500 ml water) to prevent pathogen growth. Forty to fifty neonates were placed into 5.5 oz plastic cups and incubated at 27°C (16 h photoperiod). Pupae were collected from these cups after 14-21 days and placed into one gallon glass jars with wire mesh lids for adult emergence. Adults were provided 10% sucrose solution and wax paper sheets on which to oviposit. Egg sheets were collected daily, washed in 3% formalin for 10 min., and rinsed in tap water for 10 min.

Bioassays

One pair of neonates were placed into each of 30 one ounce plastic cups containing approximately five grams of treated diet. Larvae were exposed to four to six concentrations of Cry1Ac ranging from 0.001 to 100 µg a.i./ml diet, plus a control group. Larvae where exposed to treated diet for 8-12 days, and larval weights were recorded when larvae in the control group reached the middle to end of the fifth instar. Larvae were weighed using a Sartorius balance.

Selection for Resistance

Three populations exhibiting reduced susceptibility to Cry1Ac were exposed to Cry1Ac in the laboratory in order to determine if resistance could be amplified. The selected populations were Arizona (Dome Valley), Arizona (Yuma Agricultural Center), and Florida (Belle Glade). Each strain was selected by placing 60-70 neonates on diet containing 1000 µg Cry1Ac/ml and allowing them to complete development. This procedure was carried out on three nonconsecutive generations over approximately six months.

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Data Analysis

Regression analysis of larval weights against log concentration of Cry1Ac (µg a.i./g diet) was performed to obtain slope and intercept values. I_{50}s were derived by solving for x in the following equation:

$$\text{Log}_{10}x = \frac{0.5c-b}{m}$$

where c = mean weight of controls, b = *y* intercept, and m = slope.

Results

Susceptibility to Cry1Ac

Susceptibility to Cry1Ac, as measured by I_{50}s, ranged from 0.0477 µg/ml for the laboratory reference strain to 4.31 µg/ml for AZ (Yuma Agricultural Center). This difference translated into a 90-fold reduction in susceptibility to Cry1Ac (Figure 1; Table 1). The two foreign populations, Spain and Thailand, with I_{50}s of 0.077 and 1.17 µg/ml, respectively, were 1.6 and 24 times less susceptible to Cry1Ac than the laboratory reference strain. The four least susceptible populations evaluated, Arizona (Dome Valley), Arizona (Roll), Arizona (Texas Hill), and Arizona (Yuma Agricultural Center), were collected from an area of intensive vegetable production in southwestern Arizona.

Response to Selection With Cry1Ac

All three selected strains had individuals capable of completing development on diet treated with 1000 µg Cry1Ac/ml diet. However, only two of the three selected strains yielded beet armyworms that were substantially less susceptible to Cry1Ac. I_{50}s increased from 2.49 to 7.42 µg Cry1Ac/ml diet for the Arizona (Dome Valley) strain, from 4.31 to 5.69 for the Arizona (Yuma Agricultural Center) strain, and from 2.43 to 17.4 for the Florida (Belle Glade) strain. These represent 3.0, 1.3, and 7.2-fold responses to selection, respectively (Table 1). Therefore, both Arizona and Florida beet armyworm showed evidence of genetically-based resistance to Cry1Ac, as evidenced by a response to selection. These two selected strains were 50 and 120 times less susceptible to Cry1Ac than were the more susceptible of Arizona field populations (e.g., Upper Gila Valley) and 160 to 360 times less susceptible to Cry1Ac than was our reference strain, USDA (Table 1).

Discussion

We found large differences in susceptibility of beet armyworm to Cry1Ac. Based on contrasts of I_{50}s, non-selected strains fell into four general susceptibility categories. Four strains had I_{50}s that were <4-fold higher than the reference strain. Two strains had I_{50}s that were 25- to 27-fold greater than the reference strain. Five strains had I_{50}s that were 46- to 63-fold greater than the reference strain. Lastly, one non-selected strain, collected in Yuma, Arizona, was 90-fold less susceptible than was the reference population. Vegetables are intensively grown in this area and subject to periodic uses of foliar Bt treatments. Also notable is that deployment of Bt cotton in the Yuma area has been extremely limited. Thus, the least susceptible of all (non-selected) populations evaluated came from Arizona and was from an area of the state in which the use of Bt cotton has been extremely limited.

The Florida (Belle Glade) and Arizona (Dome Valley) strains were heterogeneous with respect to a gene or genes that conferred resistance to Cry1Ac. Selection reduced susceptibility of the Belle Glade strain by 7-fold and the Dome Valley strain by 3-fold. These resistant strains were 160- to 360-fold less susceptible to Cry1Ac than was the reference strain. Thus we have isolated intense resistance to Cry1Ac in two strains of beet armyworm from two different states. Future work will explore the inheritance of resistance to Cry1Ac and characterize the relationship between regional use of Bt cotton versus susceptibility of beet armyworm.

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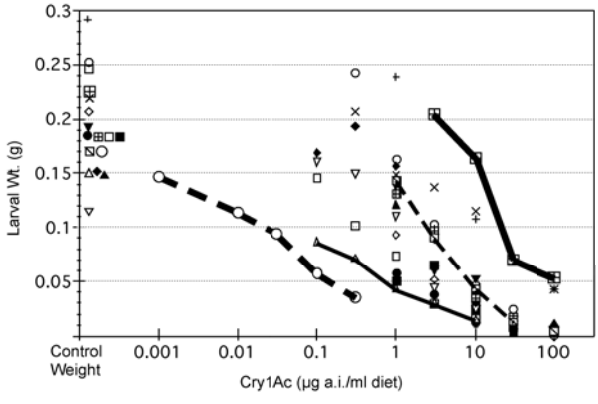

Figure 1. Larval weights of beet armyworm after 8-12 day exposure of neonates to Cry1Ac-treated diet. Shown from left to right are: laboratory reference strain (USDA), most susceptible field strain (AZ-Upper Gila Valley), least susceptible field strain (AZ-Yuma Agricultural Center), and most resistant selected population, (FL-Belle Glade Bt selected). See Materials and Methods for details of bioassays.

Table 1. Susceptibility of beet armyworm to Cry1Ac as measured by I_{50}s. I_{50} is defined as the amount of Cry1Ac that resulted in a 50% reduction in larval growth from the first to fifth instar (27°C and a 16 hr. photoperiod).

Population	I_{50} µg Cry1Ac/ ml diet	RR
USDA	0.0477	1.0
Spain	0.0772	1.6
AZ(Upper Gila Valley)	0.151	3.2
AZ(Stanfield)	0.154	3.2
AZ(Maricopa)	0.181	3.8
Thailand	1.17	25
AZ(Sommerton)	1.29	27
AZ(Parker-McCabe)	2.23	47
FL(Belle Glade)	2.43	51
AZ(Roll)	2.46	51
AZ(Dome Valley)	2.49	52
AZ(Texas Hill)	3.01	63
AZ(Yuma Agric. Ctr.)	4.31	90
AZ(Yuma Agric. Ctr.) Bt-sel.	5.69	120
AZ(Dome Valley) Bt-sel.	7.42	160
FL(Belle Glade) Bt-sel.	17.4	360