LEPIDOPTERAN ADULT FEEDING DISRUPTION TEST (FDT) TO DETECT INSECTICIDE RESISTANCE

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Abstract

Newly emerged male susceptible lab-strain *Heliothis virescens*, tobacco budworm (TBW) moths were fed increasing concentrations of permethrin in dyed (blue) sucrose. The permethrin concentration that resulted in 100% mortality in 24 hours was shown to have a complementary visual marker for feeding disruption (minimal amount of blue feces relative to controls with no insecticide). When resistant TBW moths were fed the diagnostic dose, they produced abundant dyed feces relative to susceptible moths. Proof-of-concept was demonstrated for an adult FDT in which resistant moths can be distinguished from susceptible moths on the basis of their ability to feed on insecticide in a dyed sucrose solution and produce a visually distinct amount of dyed feces. An FDT for moths is an alternative to the adult vial test (AVT) that will allow testing of insecticides with ingestion as well as contact activity. The adult moth FDT test has several additional advantages including ease of mass production, an easy to read assay end point and a potentially short assay time.

Introduction

The development of resistance to insecticides has been documented since 1914 (IRAC, 2005). Resistance to inorganic insecticides, organic insecticides and Bt sprays has developed within 2–20 years of being applied to populations of target insects (IRAC, 2005). Looking at the tobacco budworm, *Heliothis virescens*, in cotton as a case in point, populations of this insect have developed resistance to a succession of four classes of insecticides since the 1960s (Sparks, 1981; Elzen et al., 1992)

The prospect of resistance as well as restrictions on the development of new insecticides has made the efficacy of any insecticide a resource to be stewarded. Insect Resistance Management (IRM) programs have been developed and implemented by stakeholders to preserve insecticide efficacy (US EPA, 2001). The role of resistance-monitoring assays in these programs is to assess the success of IRMs to maintain insecticide efficacy. Types of resistance-monitoring assays include biochemical and nucleic acid assays and bioassay (Abdel-Aal et al., 1993). Biochemical and nucleic acid assays are based on elucidated resistance mechanisms and involve the analysis of homogenates of whole body or specific tissues. They can be used to confirm that a known resistance mechanism is present in a population and is potentially associated with a reduction in insecticide effacacy; and, they can be used to measure the initial frequency of resistance genes or alleles before a new insecticide technology is introduced (Yu, 2008). Bioassays on the other hand, use the intact (live) insects and thus measure susceptibility based on any and all resistance mechanisms, identified or not.

The prevailing bioassay for resistance-monitoring of moths is the adult vial test (Plapp et al., 1987). It takes the form of treating the insides of vials with an insecticide, adding a test insect (moth), and assessing mortality over time. The AVT has several limitations: (i) is limited to insecticides with contact activity, (ii) susceptibility may be affected by a lack of food or dehydration during the test period; (iii) specialized equipment is required to prepare the vials with a precise and consistent dose of insecticide; and (iv) the method of exposure to the insecticide and the mortality endpoint can extend the assay time.

In the case of larval Lepidoptera, a resistance-monitoring bioassay based on feeding disruption has been developed for chemical and Bt protein insecticides (Bailey et al., 1998, 2001; Roe et al., 2000, 2003, 2004, 2005). The assay format is an array of rehydrateable (artificial diet) meal pads containing a blue indicator dye. At the time of testing, the meal pad is rehydrated and loaded with a diagnostic dose of insecticide (alternatively the insecticide can be pre-loaded before hydration). The diagnostic dose distinguishes resistant from susceptible insects based on their ability to feed on the insecticide-loaded meal pad with feeding measured by the production of dyed (blue) fecal pellets.

The feeding of susceptible larvae is disrupted by the insecticide and results in the minimization or elimination of fecal production. In contrast, the feeding of resistant larvae is not disrupted, and fecal production proceeds throughout the test period. Blue feces accumulate on the white background of the FDT plate .

A feeding-disruption assay for caterpillars having been developed for a variety of applications, the prospect of developing such an assay for adults was examined in the current paper. After determining that moths would feed on a dyed sucrose solution and produce dyed feces, the objectives of the work summarized here were to determine whether moths would feed on an increasing concentration of insecticide in dyed nectar and show a mortality dose response; whether a dose response for reductions in blue fecal production can also be obtained and positively correlated with increased mortality; and whether the assay could be validated with a resistant TBW strain.

Materials & Methods

Insects

Susceptible and resistant lab strains of the tobacco budworm (TBW), *Heliothis virescens*, were reared at North Carolina State University from eggs under controlled conditions. Unfed newly emerged male moths were used for the studies that follow.

Test Arenas

Test arenas for these proof-of-concept experiments consisted of 1-gallon white plastic food tubs with the open end covered with plastic food wrap. Each tub contained a feeding platform consisting of an inverted 4" x 2" styrofoam food cup in the bottom of which holes had been cut with a # 6 cork borer. Five Eppendorf tubes (2.0-ml volume each) were inserted into the holes in the styrofoam cup bottom. The Eppendorf tubes held the test solutions.

Test Solutions

Dyed nectar consisted of a 10% sucrose solution containing 0.08 mg/ml trypan blue dye. Dyed insecticide solutions consisted of different concentrations of Pounce® (3.2 EC, 38.4% permethrin) diluted in the dyed nectar. Control solutions were the dyed nectar without insecticide.

Holding Conditions

Test arenas were held in a growth chamber at 27 ± 1° C, 65% relative humidity and a light:dark cycle of 14 hours light:10 hours dark.

Assay Procedure

For each test arena, five unfed newly emerged male moths (0 – 24 hours in age) were immobilized by chilling at 4° C and transferred to the feeding tub with flat forceps. The arenas were held in a growth chamber for 24 hours before observations were made.

Observations

After 24 hours of feeding, mortality and production of blue feces were measured. Mortality was measured in terms of numbers of moths that were ecologically dead: Those unable to demonstrate coordinated movement in response to being prodded with forceps. For the diagnostic dose, the 24-hour amount of dyed fecal production was visually assessed relative to the amount produced by moths that fed on the control solutions.

Statistical Analyses

SAS Proc Univariate (SAS Institute, Cary, NC) was used to calculate means and the standard errors of the mean (SEM).

Results and Discussion

Mortality results for lab-strain susceptible moths fed permethrin in dyed sucrose for 24 hours are presented in Table 1. There was no mortality in the controls and the lowest concentration of permethrin. However, as permethrin concentration increased from 2.5 – 20.0 µg/ml, mortality increased from 30% to 100% of the tested moths. On the basis of these results for the susceptible strain, the 20.0 µg/ml permethrin solution was chosen as the diagnostic dose.

Table 1. Twenty-four hour mortality of newly-emerged lab-strain susceptible male *H. virescens* moths fed permethrin in dyed sucrose solution.

Permethrin concentration in dyed sucrose	Mean mortality	Standard error of mortality mean	n*
(µg/ml)	(%)	(%)	
0.00	0	0	9
1.25	0	0	4
2.50	30	19.1	4
5.00	65	15	4
10.00	80	11.5	4
20.00	100	0	5

*n = number of tests (five moths per test)

A representative view of the amount of dyed feces produced by lab-strain susceptible moths fed the permethrin diagnostic dose for 24 hours is presented in Fig. 1 (right). The amount of blue feces produced during the test period at the diagnostic dose was minimal with only a few small spots visible. In contrast, the amount of blue feces produced by moths feeding on the control solution with no insecticide was abundant and visually distinct.

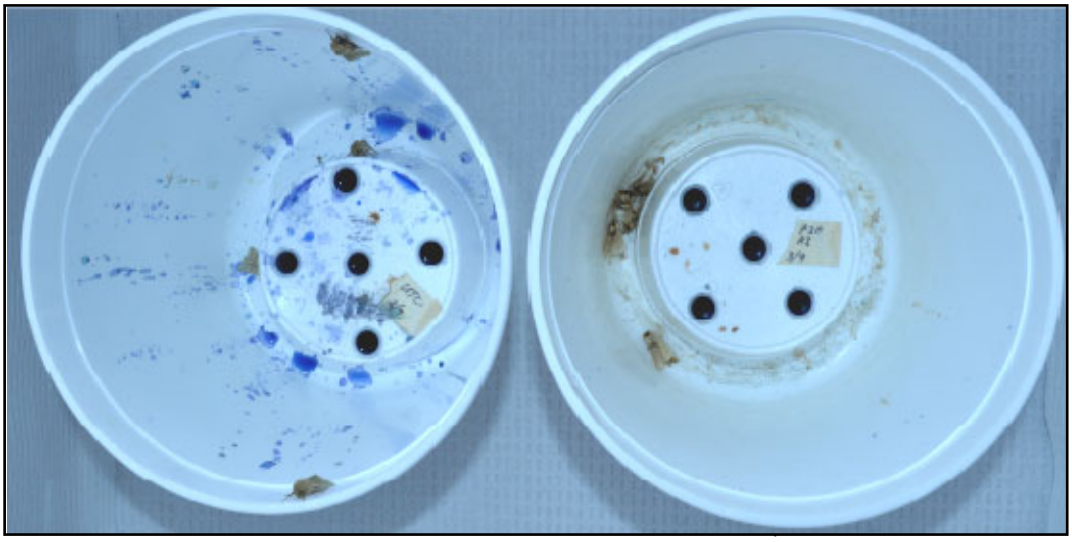

Fig. 1. Dyed feces produced by five newly-eclosed susceptible TBW moths fed the diagnostic dose of permethrin (20 µg/ml) in dyed nectar (tub on right) for 24 hours. Control treatment (dyed nectar without insecticide) is tub on left.

Mortality results for both resistant and susceptible lab strains of the TBW moths fed the permethrin diagnostic dose is presented in Table 2. Mortality of both susceptible and resistant moths feeding on the control solution (dyed sucrose without insecticide) was 0%. However, mortality of the susceptible moths that fed on the diagnostic dose was 100% while that of resistant moths was 0%.

Table 2. Twenty-four hour mortality of newly-emerged lab-strain susceptible and resistant male t *H. virescens* moths fed the diagnostic dose of permethrin in dyed sucrose solution.

TBW strain	Permethrin concentration in dyed sucrose	Mean mortality	Standard error of mortality mean	n*
	(µg/ml)	(%)	(%)	
Susceptible	0	0	0	3
Susceptible	20	100	0	3
Resistant	0	0	0	2
Resistant	20	0	0	2

*n = number of tests (five moths per test)

A typical example of the amount of dyed feces produced by lab-strain resistant moths fed the permethrin diagnostic dose for 24 hours is presented in Fig. 2 (tub on right). The control response was as expected, i.e., abundant blue fecal production (Fig. 2, left) The resistant strain also showed abundant blue fecal production Fig. 2, right) and distinct from susceptible moths (Fig. 3).

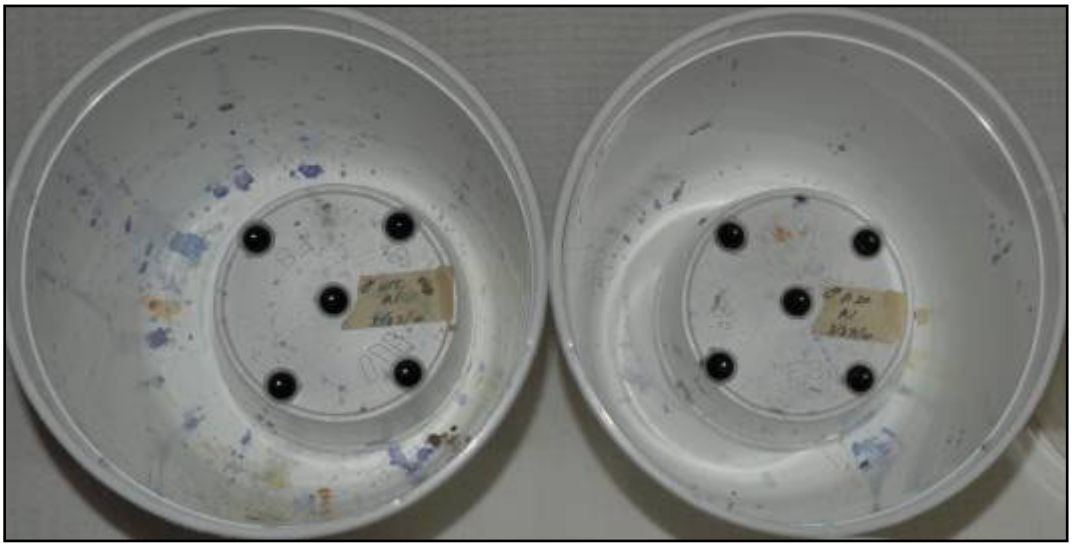

Fig. 2. Dyed feces produced by five newly-emerged resistant TBW moths fed the diagnostic dose of permethrin (20 µg/ml) in dyed nectar (tub on right) for 24 hours. Control treatment (dyed nectar without insecticide) is tub on left.

Fig. 3. Contrast in the amount of dyed feces produced by five newly-emerged susceptible (left tub) and resistant (right tub) TBW moths fed the diagnostic dose of permethrin (20 µg/ml) in dyed nectar for 24 hours.

Summary

Newly-emerged male susceptible lab-strain TBW moths were fed increasing concentrations of permethrin in dyed sucrose. The permethrin concentration that resulted in 100% mortality in 24 hours resulted in minimal blue feces production and is a marker for susceptibility. When resistant TBW moths were fed the diagnostic dose, they produced abundant dyed feces relative to the susceptible moths. Proof-of-concept was demonstrated for an adult FDT in which resistant moths can be distinguished from susceptible moths on the basis of ability to feed on insectide in dyed sucrose solution and produce dyed feces.

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