CHALLENGES FOR REARING OF THE STINK BUG: SUCCESSES, FAILURES, AND RNAi SCREENING

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Abstract

In order to evaluate the efficacy of using an artificial diet to establish and maintain a stink bug colony, adult *Euschistus servus* (Say), brown stink bugs, were collected from soybeans near Plymouth, NC and fed either pod soybeans or NI diet (Cohen, 2000). Preliminary results of this recently-initiated and on-going study are presented. Survival of the parentals was 75 days on NI diet vs. 23 days on pod soybeans. Of the F1 2nd instars placed on soybeans, 9.7% succeeded in developing to the adult stage vs. 6.3% on NI diet. Development time of the F1s (2nd instar to adult) was 30.0 days on soybeans vs. 42.5 days on NI diet. The longevity of the F1 males that developed on NI diet was 37.0 days vs. 4.5 days on soybeans. One female developed on soybeans and none on NI diet. This effort has been undertaken in the context of a parallel effort to identify an artificial diet suitable for feeding studies to screen dsRNA constructs for insecticidal efficacy against stink bugs.

Introduction

A consequence of the low-chemical-spray environment that has resulted with the success of the boll weevil eradication program and the widespread adoption of Bt cotton has been emergence of plant bugs and stink bugs to become major pests of cotton (Leonard, 2008). Recent advances in DNA sequencing technology have provided the opportunity to identify potential hemipteran genes as targets for RNA interference (RNAi) and the prospect of transgenic alternatives to Bt transgenes for the control of plant bugs and stink bugs in cotton (Roe et al., 2009; van Kretschmar et al., 2010). RNAi disrupts gene expression, in the cell cytoplasm of target organisms at the gene-transcript (messenger RNA (mRNA)) level (Huvenne and Smagghe, 2010). The agent of RNAi is dsRNA (double-stranded RNA). The mechanism by which this disruption occurs is the cleavage of introduced dsRNA into siRNAs (short-interfering RNAs) which are complementary in their nucleotide sequence to the targeted insect mRNA. These complementary siRNAs interact with the target mRNA and prevent it from being translated into proteins that are essential to the physiology of the pest organism (Huvenne and Smagghe, 2010).

The efficacy of transforming plants to express dsRNAs for pest control has been demonstrated with two non-hemipterans. Corn, *Zea mays*, has been transformed with transgenes expressing dsRNA that reduced expression of a target gene in the western corn rootworm, *Diabrotica virgifera virgifera* (Baum et al., 2007). Mouse-ear cress, *Arabidopsis thaliana,* and tobacco, *Nicotiana tabacum,* have been transformed to express dsRNA that reduced expression of a gene in the cotton bollworm, *Helicoverpa armigera* (Mao et al., 2007).

The strategy of using RNAi for plant bug and stink bug control in cotton requires the identification of messages in the target pest that when suppressed, will result in reduced damage to the plant. Two major challenges to identifying dsRNA constructs with insecticidal efficacy are the availability of colonies of test insects and the availability of a means to efficiently feed candidate dsRNA constructs to insects to screen their insecticidal activity. This lab has previously demonstrated the feasibility of feeding chemical insecticides to plant bugs in artificial diet (van Kretschmar et al., 2009) as well as delivering both protein and chemical insecticides to Lepidoptera in hydrateable, artificial diet (Bailey et al., 1998, 2001; Roe et al., 2002). These prior successes suggest the prospect of screening double-stranded nucleic acid constructs using a similar approach. A precondition is that any artificial diet that might be used to screen dsRNA constructs targeting plant bugs or stink bugs in feeding studies must be optimal for the growth and development of the insect. What is also needed is an artificial diet for laboratory rearing of stink bugs for dsRNA screening and research on this pest in general. The objective of our on-going work here is to examine the efficacy of using an artificial diet for stink bugs.

Materials and Methods

Insects
Brown stink bug (BSB), *Euschistus servus* (Say), adults were collected with a sweep-net from an untreated soybean field near Plymouth, North Carolina (USA) in September 2010. Collected insects were contained in cheesecloth-lidded 5-gallon plastic buckets containing soybean pods from the collection site for 24 hours prior to being transferred to arenas containing test diet.

Diet
The artificial diet tested was the NI diet of Cohen (2000) wrapped in stretched Parafilm®. The reference diet was organic pod soybeans obtained frozen from a local grocery store (365™ brand blanched soybeans in natural shell, Whole Foods Market, Austin, TX). NI diet and thawed soybeans were replaced in the feeding arenas every two days.

Feeding Arenas for Field-Collected Parentals
Twenty-four hours after collection, 14 adults (6 females and 8 males) were transferred to 1-gallon plastic tubs lidded with cheesecloth. One arena for each diet was prepared. The water source was distilled water in a 50-ml flask with a cotton dental wick. Oviposition substrates were strips of cheesecloth.

Hatching Arenas for F1 Eggs
Egg masses produced by the adults described above were transferred on the oviposition cheesecloth to 9-cm plastic Petri dishes containing a filter paper bottom and a moist cotton dental wick. One egg mass was transferred to each dish. After hatching and molting, F1 2nd instars (N2s) were transferred using a fine-tipped brush to the arenas described below.

Feeding Arenas for F1 N2s
N2s were transferred to individual (1 N2 per dish) 9-cm Petri dishes containing either of the two diets plus a moistened cotton dental wick. Thirty-two N2s were transferred to dishes containing NI diet and 31 N2s were transferred to dishes containing soybeans.

Holding Conditions
Feeding arenas were held in a growth chamber at 27 ± 1° C, relative humidity = 50% and a light:dark cycle = 16 hours light: 8 hours dark.

Observations
Daily observations were made as follows: For parentals, egg production and survival; for F1 nymphs, developmental stage duration (number of days as N2, N3, N4 and N5) and developmental success (number of N2s that succeeded in becoming adults); and for F1 adults, gender, body weight at eclosion and longevity. The lack of F1 synchrony and the death of F1 females prevented mating and an evaluation of F1 fecundity.

Statistical Analyses
SAS Proc Univariate (SAS Institute, Cary, NC) was used to calculate means and standard errors of means (SEMs). SAS Proc t-Test was used to test for differences between diet for the F1 nymphal duration of stage and F1 adult body weight and longevity ($\alpha = 0.05$).

Results and Discussion

Survival times for the 14 field-collected brown stink bug parental adults transferred to soybeans or NI diet appear in Fig. 1. Numbers of BSBs placed on soybeans declined rapidly, and less than 50% remained by day 9; the last survivor was dead on day 59. All BSBs placed on NI diet were alive at day 6 and 50% remained at day 35. The last survivor on NI diet was dead on day 86. The parental females transferred to soybeans produced 5 egg masses within four days of the transfer but did not produce eggs thereafter. No eggs were produced by the females transferred to NI diet.

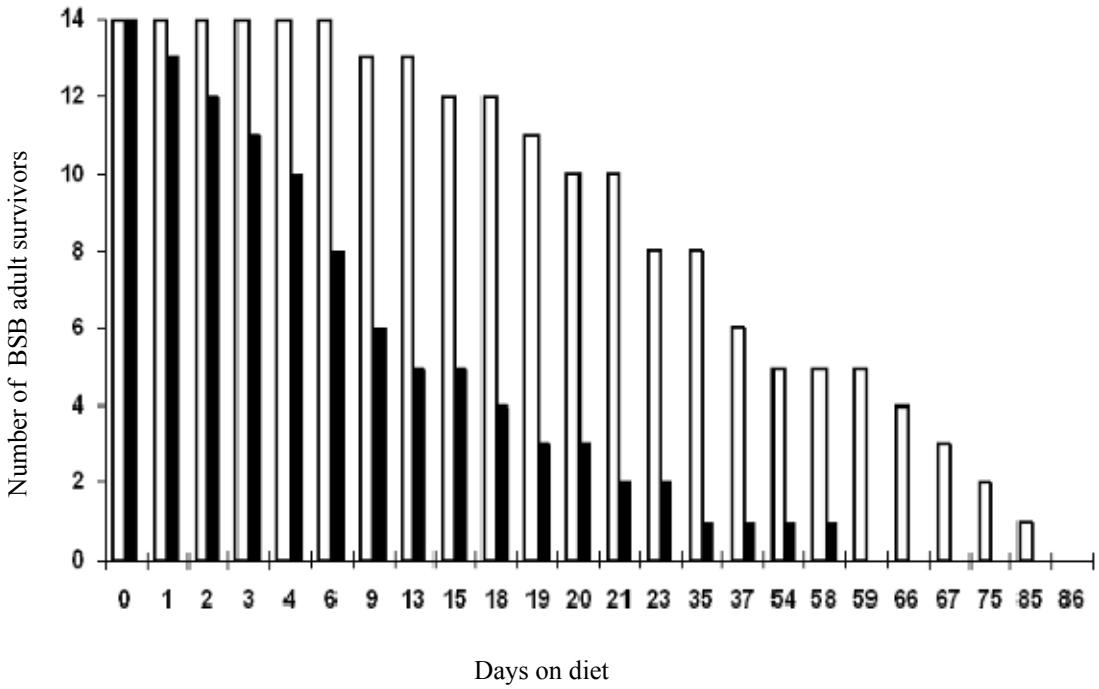

Fig. 1. Survival time of 14 field-collected brown stink bug (BSB) parentals (males & females) transferred to soybeans (black bars) or NI diet (white bars). The last adult on soybeans was dead at day 59 vs. day 86 on NI diet.

Fig. 2 shows survival times for the field-collected parental females and males. On soybeans, the last opportunity for mating occurred at day 23 when the last male alive could have mated with two surviving females. On NI diet, the last opportunity for mating occurred at day 75 when the last female alive could have mated with two surviving males.

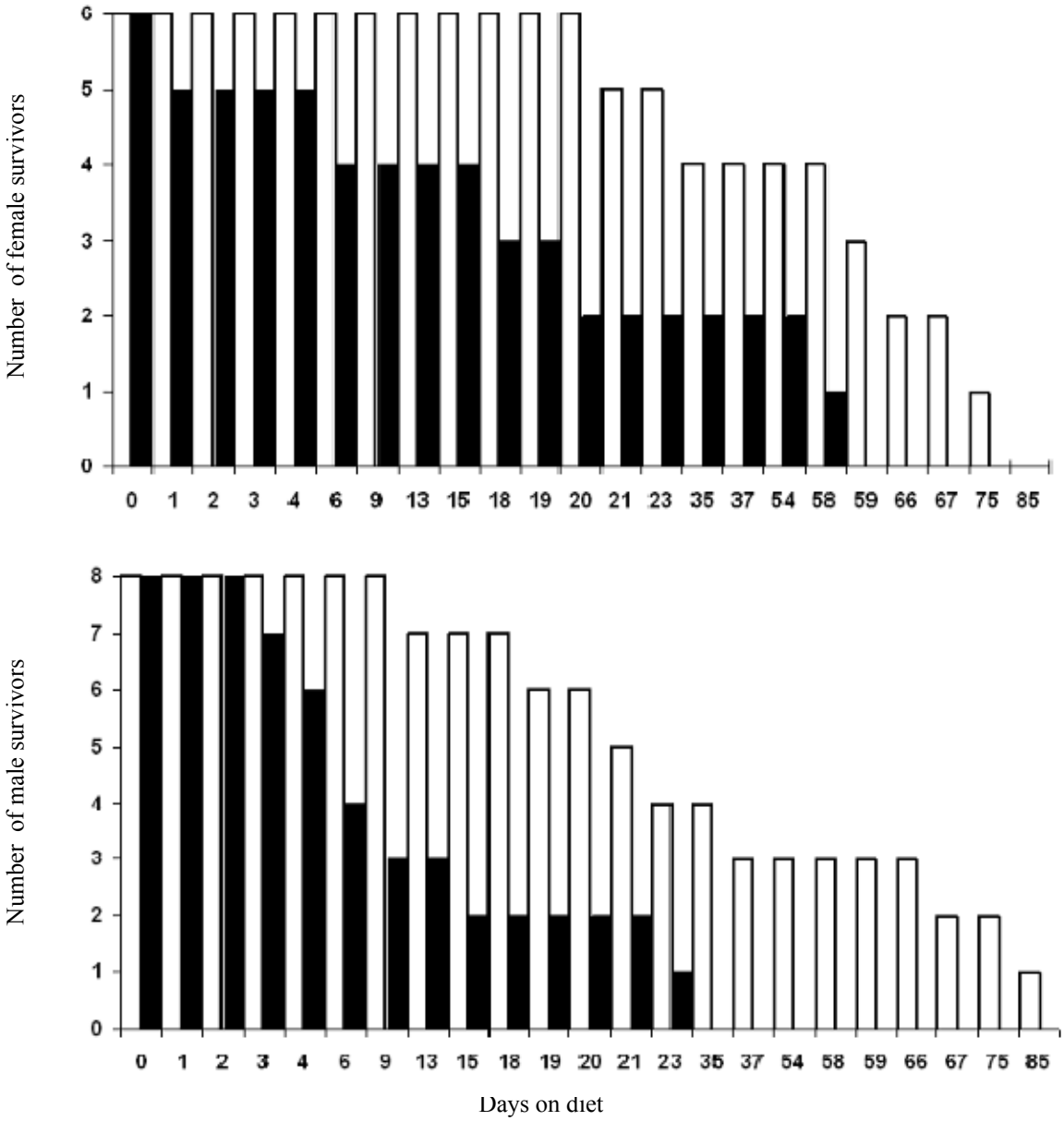

Fig. 2. Results for field-collected parental brown stink bug adults by sex. Survival time for 6 females (top) and 8 males (bottom) transferred to soybeans (black bars) or NI diet (white bars). The last female on soybeans was dead at day 59 vs. day 76 on NI diet. The last male on soybeans was dead at day 24 vs. day 86 on NI diet.

The developmental stage duration of F1 N2 (2nd instar) nymphs placed on soybeans or NI diet is presented in Fig. 3. Mean total development time (N2 through N5 to F1 adult males) on soybeans was 30.0 days vs. 42.5 days on NI diet (P = 0.04; t-test). The single F1 female to develop on either diet developed on soybeans in 19 days.

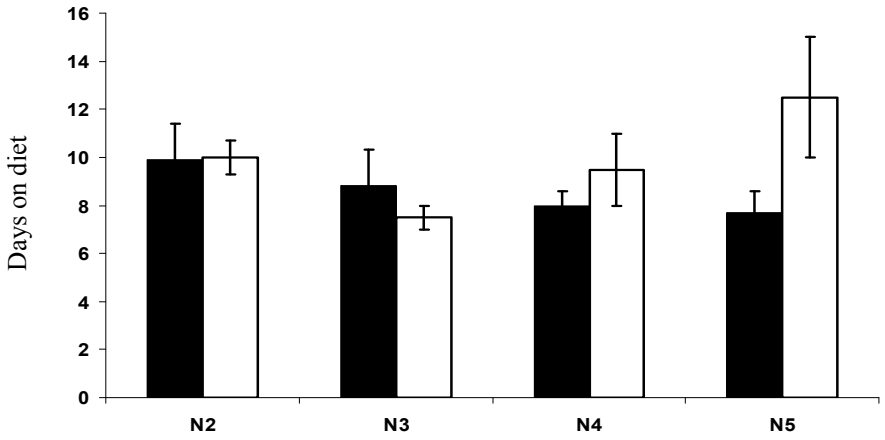

Fig. 3. Duration of each stadium for nymphs fed NI diet (white bars) or soybeans (black bars) while developing from N2 (2nd instar) through N5 to the adult stage. Initially, 32 N2s were placed on NI diet and 31 N2s on soybeans. Mean total development time (N2 to adult males) on soybeans was 30.0 days vs. 42.5 days on NI diet (P = 0.04; t-test). The single F1 female to develop on either diet developed on soybeans in 19 days.

The developmental success of F1 N2s placed on soybeans or NI diet is presented in Fig. 4. Of the 31 N2s placed on soybeans, 3 (9.7%) developed into F1 adults. Of the 32 N2s placed on NI diet, 2 (6.3%) succeeded in developing into adults.

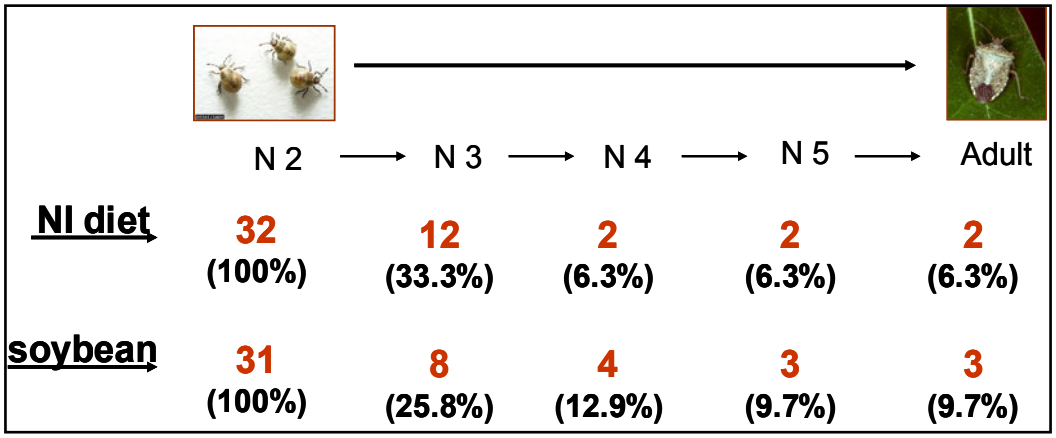

Fig. 4. Developmental success of N2s feeding on NI diet or soybeans. Numbers are of individuals on either diet. Percents are based on numbers of individuals at each instar/stage relative to the number of N2s initially placed on diet.

Characteristics of the F1 adults developed on either diet is presented in Table 1. Two males and one female developed on soybeans; the two adults that developed on NI diet were both males. The mean body weight of males that developed on soybeans was 0.1216 g vs. 0.0973 g for the males that developed on NI diet ($P = 0.27$; t-test). However, the mean longevity of the males on soybeans was only 4.5 days vs. 37.0 days for the males on NI diet ($P = 0.02$; t-test). The female that developed on soybeans lived 19 days.

Table 1. Sex ratios, longevity and body weight at eclosion of F1 brown stink bug adults developed from N2 nymphs transferred to soybeans or NI diet.

Diet	Sex	n	Mean longevity ± SEM (days)	Mean body wt. ± SEM (grams)
NI	M	2	37.0 ± 5.0a	0.0973 ± 0.0031a
	F	0	-	-
Soybeans	M	2	4.5 ± 5.0b	0.1261 ± 0.0159a
	F	1	(19 days)	(0.0873 g)

Means in columns with the same letter were not significantly different ($P \leq 0.05$)

Summary

Adult brown stink bugs, *Euschistus servus* (Say), were collected from a soybean field near Plymouth, NC and transferred to either pod soybeans or NI diet to evaluate their use for the laboratory rearing of these insects. Survival of the parentals measured in terms of a last opportunity for a mating was 75 days on NI diet vs. 23 days on pod soybeans. Only the adults transferred to pod soybeans after collection produced eggs; however, this egg production occurred within 4 days of the transfer and is probably not a measure of the contribution of pod soybeans vs. NI diet to egg development. Of the F1 N2s placed on soybeans, 9.7% succeeded in developing to the adult stage vs. 6.3% of those placed on NI diet. Development time of the F1s (from N2 to adult) was 30.0 days on soybeans vs. 42.5 days on NI diet. The body weight of F1 adult males that developed on soybeans was 0.13 g vs. 0.10 g on NI diet. The longevity of the F1 males that developed on NI diet was 37.0 days vs. 4.5 days on soybeans. The only female to develop on either diet developed on soybeans and lived for 19 days. These preliminary results based on a last field-generation of brown stink bugs for the growing season in North Carolina do not eliminate the possibility of using the NI diet to establish and maintain a BSB colony and the use of this diet for RNAi screening and as a feeding disruption test for insecticide susceptibility. Survival time of the parental adults and longevity of the F1 adults were both longer on NI diet than on soybeans.

Acknowledgements

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