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# Effect of Diet on Mortality and Larval Duration in Chrysodeixis includens (Lepidoptera: Noctuidae)


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# EFFECT OF DIET ON MORTALITY AND LARVAL DURATION IN *Chrysodeixis includens* (Lepidoptera: Noctuidae)

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## ABSTRACT

Diet mediates many life history trade-offs and therefore is an important environmental component to consider. Because some organisms share food sources with humans, they encounter anthropogenic changes to their diet, such as genetically modified crops and chemical applications, which can contribute to additional complexity in their environment. To understand the effect of diet on the life history of the soybean looper, *Chrysodeixis includens,* we exposed larvae to several different food sources, including genetically modified soybean leaves and the pesticide spinosad. Significant differences in larval instar duration and timing of larval mortality among treatments were observed indicating that type of diet has complex effects on the life history of *C. includens*.

***Keywords:*** *Chrysodeixis includens*, soybeans, spinosad

## INTRODUCTION

Diet is an important part of the life history of organisms because it mediates trade-offs between major factors such as size, reproduction, mortality, and immune response (Gross and Newberne 1980; Clancey et al. 2002; Mason et al. 2016). The diets of insect pests that eat plants also used by humans are particularly interesting because of the economic importance of the crops and the additional anthropogenic factors that the insects encounter, such as genetically modified (GM) crops and the application of agricultural chemicals.

One such insect pest in the southern United States is the soybean looper, *Chrysodeixis includens*, a lepidopteran species that spends its larval stage feeding on plant leaves. *Chrysodeixis includens* feeds on approximately 30 different plant species, including, but not limited to soybeans, cotton, and sweet potatoes (Kid and Orr 2001). The primary food source of *C. includens* is soybean foliage, but outbreak populations of *C. includens* on soybeans can cause additional damage, especially in southern states where cotton and soybeans are grown together (Higley and Boethel 1994; NCSU 2016). Because of the potentially complex interaction of this insect pest with its environment, investigating factors such as diet, that affect the life history of *C. includens*, can contribute to our understanding of the ecological integrity of certain agricultural systems.

To better understand how diet affects *C. includens*, we exposed larvae to different diets with and without a pesticide. Specifically, we reared *C. includens* on a conventional strain of soybeans, a soybean strain modified for glyphosate resistance, since these are commercially available in the United States, and an artificial insect diet commonly used for populations reared in the lab. We also exposed *C. includens* to the organic class pesticide spinosad, which is a relatively new pesticide used against a variety of pests including *C. includens* (Mascarenhas and Boethel 2000; Cossentine et al. 2012; Olmstead and Shelton 2012). Spinosad is a neurotoxin that can have either oral or contact toxicity (Pineda et al. 2004). We measured the length of time larvae spent in each instar (stadium) and mortality of *C. includens* since it is the larval stage of this species that feeds on soybean foliage.

## MATERIALS AND METHODS

### Soybean Cultivars

A conventional cultivar (CLOJ095-4) was obtained through the USDA Soybean Germplasm Collection. This is a cultivar without tolerance to glyphosate herbicide. It was developed for the non-GMO soybean market (USNPGS 2016). A genetically modified cultivar, RoundUp Ready, was also obtained through the USDA. This cultivar is resistant to glyphosate and is commercially available. Soybean seeds were planted in medium clay pots with Miracle Grow potting mix and grown in an environmental chamber with water added when soil felt dry to the touch. Approximately 10 plants of each cultivar were kept throughout the experiment and leaves were excised as needed for treatments.

### Rearing

*Chrysodeixis includens* pupae were obtained from a lab-reared population held in continuous culture at the University of Georgia for at least 15 years as described in Strand (1990). Field-captured individuals have been periodically introduced into this population over this time period, which contributes to genetic diversity. Pupae were reared in 0.95 L plastic deli containers containing approximately 20 pupae each. Deli containers contained paper towels to provide egg-laying substrate, and moths were fed using a cotton ball soaked in 10% sucrose solution, which was replaced daily. Egg sheets were checked daily and held until first instars emerged, at which point they were haphazardly assigned to one of six experimental treatments described below. Each *C. includens* was reared at 27 $^{o}$C, 16:8 (L:D), and 70% relative humidity in a Percival biological incubator.

### Experimental Setup

Single first instar larvae were transferred to individual 100 x 15-mm plastic petri dishes and were assigned to one of six treatments: artificial diet (n = 112), GM soybean leaves (n = 106), conventional soybean leaves (n = 112), artificial diet with spinosad at the manufacturer's recommended concentration of 78 ppm (n = 41), conventional soybeans with spinosad applied at 78 ppm (n = 47), and GM soybean leaves with spinosad applied at 78 ppm (n = 29). Larvae assigned to artificial diet were fed ad libitum on 2 x 2-cm pieces of artificial corn meal diet (Southland Products Incorporated) prepared in the lab. Spinosad was applied to artificial diet using an overlay assay with a final concentration in the food of 78 ppm (Mascarenhas and Boethel 2000). Soybean leaves were excised and placed into plastic petri dishes containing *C. includens* larvae and replaced every 24 hours according to Thomas and Boethel (1993). Approximately 0.6 ml of spinosad at 78 ppm was applied to excised GM and conventional soybean leaves by spraying the leaves with a small spray bottle from approximately 7 cm from the leaf. Spinosad was applied on the first day of the third instar because some younger larvae drowned in the pesticide, confounding measures of larval mortality and resulting in a lower sample size for these cohorts. All other treatments began on the first day of the first instar. All caterpillars were visually assessed at approximately the same time and in the same order daily to determine stage of development based on head capsule width, and mortality based on responsiveness to touch. Percent mortality of each instar was calculated by dividing the number of larvae that died during the instar by the total number of larvae alive at the beginning of the instar. Larval duration, or stadium, was determined by counting the number of days spent in each instar. We use *stadium* to refer to a larval period followed by molting to the next instar. We use *duration* to refer to a larval period in which larvae die without molting to the next instar. All statistical analyses were completed in SPSS (v. 23).

## RESULTS

*Instar stadia vary among diets*

There was a significant difference in overall time spent as larvae, pooled across instars, among the different diets (ANOVA: $df = 5$, $F = 30.78$, $p << 0.01$, Table I). This significant effect of diet was largely due to differences between artificial diet and soybeans, even when pooled across instars. Larvae reared on artificial diet spent significantly less time as larvae than those reared on either conventional soybeans ($t = 5.61$, $p < 0.001$) or GM soybeans ($t = 6.08$, $p < 0.001$; Table I). There was no significant difference in total time spent as larvae (instars 2–5) between larvae reared on conventional soybeans and GM soybeans ($t = 0.84$, $p = 0.20$; Table I). To see if this effect of diet varied among instars, we did post hoc pairwise comparisons of diet for each instar (Table II).

***Table I.*** Average larval stadia (days ± standard deviation). Total is the average total time (days) spent as larvae in instars 2–5. Individuals that died prior to pupation were excluded. First instars were excluded because these measures could vary up to 1 day.

| | **Second Instar** | **Third Instar** | **Fourth Instar** | **Fifth Instar** | **Total** |
|---|---|---|---|---|---|
| Artificial diet | 1.86 (±0.55) | 2.19 (±1.22) | 2.30 (±1.02) | 3.29 (±0.96) | 9.71 (±2.36) |
| Conventional soybeans | 2.51 (±0.94) | 3.10 (±1.29) | 3.19 (±1.72) | 4.81 (±1.57) | 15.31 (±4.98) |
| GM soybeans | 2.72 (±1.24) | 3.30 (±1.60) | 4.03 (±2.47) | 4.56 (±1.57) | 13.72 (±5.12) |

***Table II.*** Results of post hoc tests comparing larval stadia among diets, across instars, adjusted for multiple comparisons using the Fisher's LSD method. *P* values are reported for significance. Asterisks indicate nonsignificant comparisons.

| | **Second Instar** | | **Third Instar** | | **Fourth Instar** | | **Fifth Instar** | |
|---|---|---|---|---|---|---|---|---|
| | Conventional soybeans | GM soybeans | Conventional soybeans | GM soybeans | Conventional soybeans | GM soybeans | Conventional soybeans | GM soybeans |
| Artificial diet | $p<<0.001$ | $p<<0.001$ | $p<<0.001$ | ** | $p<<0.001$ | $p<<0.001$ | $p<<0.001$ | $p<<0.001$ |
| Conventional soybeans | | ** | | ** | | $p<<0.001$ | | ** |

*Spinosad affects larval mortality and duration*

The effects of spinosad are only relevant to the third instar because spinosad was initially applied at the beginning of the third instar since some smaller caterpillars in earlier instars drowned after spinosad application. Exposure to spinosad resulted in 100% mortality in the third instar unlike mortality in other treatments (artificial diet 5.4%; GM soybeans 31.1%; conventional soybeans 27.7%; Figure 1).

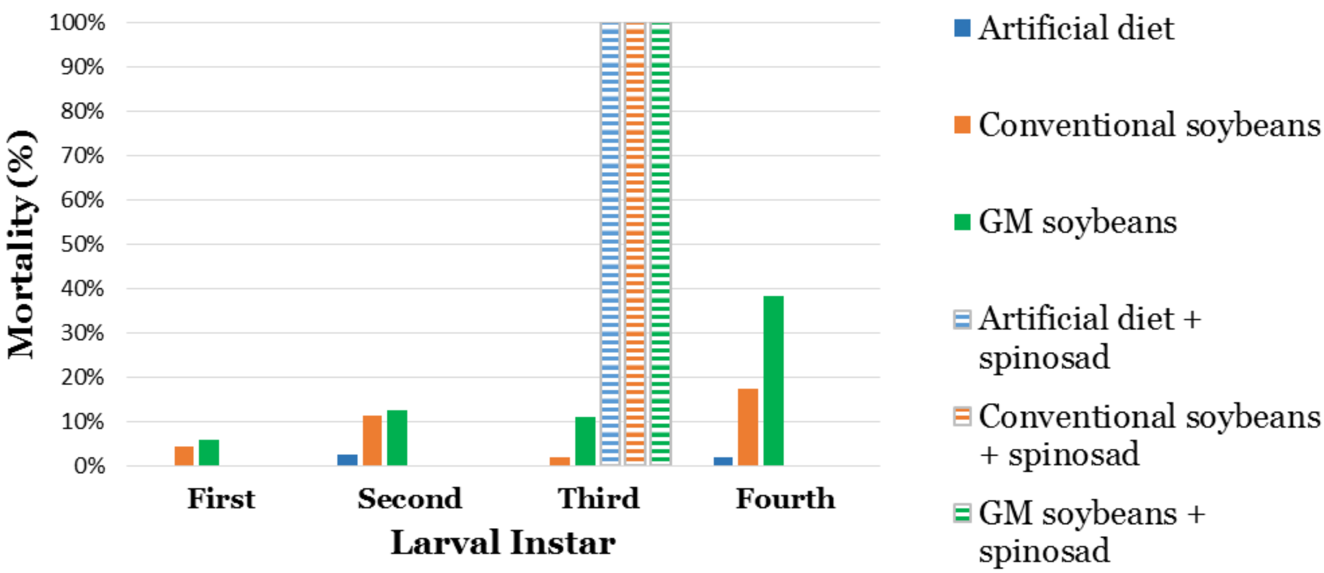


***Figure 1.*** Larval mortality for each of the first four instars expressed as a percentage. Spinosad treatments were applied on the first day of the third instar. Diet is color coded, and the horizontal striped pattern indicates spinosad exposure.

However, the duration of the third larval instar exposed to spinosad varied among diet (Figure 2, hatched bars). Larvae reared on artificial diet and exposed to spinosad lived significantly longer than larvae reared on either soybean variety and exposed to spinosad (GM soybeans + spinosad: $t = 6.5$, $p < 0.001$; conventional soybeans + spinosad: $t = 8.96$, $p < 0.001$). Additionally, in the third instar, larvae reared on artificial diet and exposed to spinosad lived significantly longer than larvae reared on artificial diet alone ($t = 3.71$, $p < 0.001$). This average extension of the third instar in larvae reared on artificial diet and spinosad relative to artificial diet alone was unlike what happens to larvae reared on soybeans. Larvae reared on conventional soybeans and exposed to spinosad spent less time as third instars than larvae reared on conventional soybeans alone ($t = 7.04$, $p < 0.001$). Similarly, larvae reared on GM soybeans and exposed to spinosad also had shorter third instars than larvae reared on GM soybeans alone ($t = 8.63$, $p < 0.001$).

## DISCUSSION

Our results show that larval life history of *C. includens* is affected by differences in diet. We presented data on larval duration and mortality for the first instar but did not follow up with statistical analysis since these results could be confounded by larval hatch

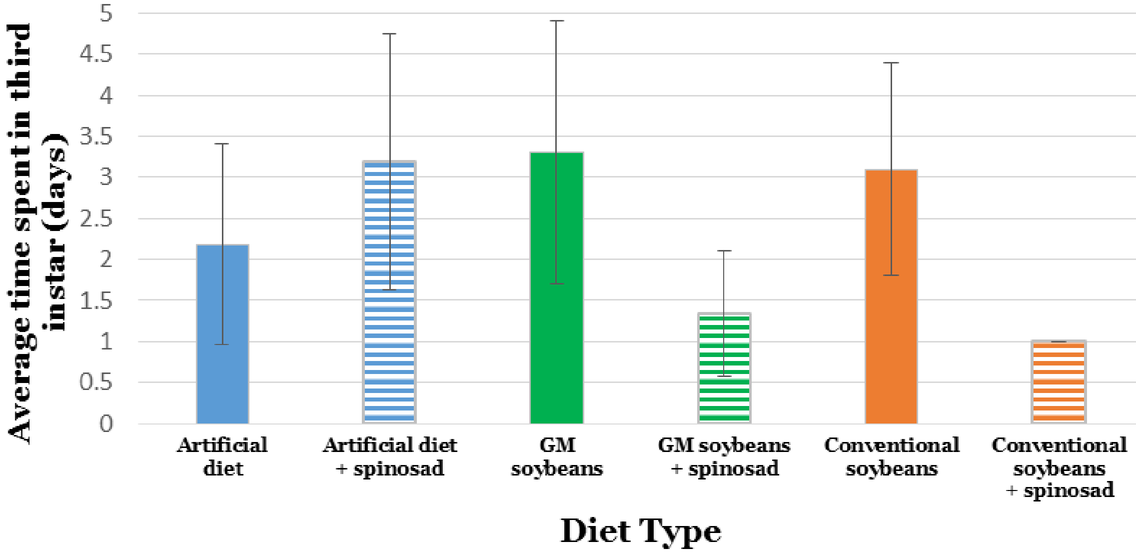


***Figure 2.*** Average time larvae spent in the third instar across all treatments (days ± standard deviation). Spinosad treatments were applied on the first day of the third instar. Diet is color coded, and the horizontal striped pattern indicates spinosad exposure.

and transfer time. Because eggs sheets were checked once daily, first instar durations could have variation of up to 24 hours.

Starting in the second instar, in the absence of spinosad exposure, larval stadia were shorter in larvae reared on artificial diet relative to either soybean variety (Table I, Table II). Similarly, in the fourth and fifth instars, after all larvae exposed to spinosad died (Figure 2), stadia were significantly shorter for larvae reared on artificial diet compared to those reared on both soybean varieties (Table I, Table II). These results are not surprising given that our population has been reared in the lab for several generations and likely somewhat adapted to this food source. Additionally, these results were likely influenced by the purposefully optimized nutrition of artificial insect diets. However, there was also a significant difference in larval duration during the fourth instar between soybean varieties, suggesting that type of soybean diet does affect larval progression.

In the third instar, when spinosad was applied, there was rapid and complete mortality of all larvae exposed to spinosad (Figure 2). This result is consistent with previous findings by Mascarenhas and Boethel (2000) showing the effectiveness of spinosad on this species. The mortality we observed was higher than Mascarenhas and Boethel (2000) reported, but we applied spinosad at the manufacturer's recommended dosage, 78 ppm, rather than at their diagnostic concentration of 60 ppm, which may explain the slight difference. Nonetheless, this work provides continued documentation of the effectiveness of spinosad against *C. includens*, at least in this population.

In the absence of spinosad, larvae reared on artificial diet had lower overall mortality than those reared on either soybean variety across all instars (Figure 1). However, larvae reared on GM soybeans had slightly higher total mortality than those reared on conventional soybeans, but the difference may not be significant and is similar in magnitude to that reported for the green clover worm, another noctuid, which showed no significant difference in larval developmental time and survivorship in larvae reared on RoundUp Ready soybeans and conventional soybeans (Morjan and Pedigo 2002).

The effect of spinosad on the third instar depended on diet. Application of spinosad to artificial diet increased the duration of the third larval instar, but application of spinosad to both soybean varieties decreased the duration of the third larval instar (Figure 2). Nonetheless, all larvae exposed to spinosad died before the fourth instar. This increased tolerance to spinosad observed when larvae were reared on artificial diet could again be due to the optimized nutrition provided by the artificial insect diet relative to the soybeans. This conclusion is anecdotally supported by observations of larval weight which suggest that those reared on artificial diet were slightly larger than those reared on both soybean varieties. Therefore, individuals reared on artificial diet may have had greater energetic reserves and were able to withstand the exposure to spinosad for a longer period of time. However, further quantitative work is needed to follow up on this observation.

Taken together, these results show that diet affects larval duration and mortality of *C. includens*. This work suggests that the variety of food sources that these insects encounter, due to development of agricultural landscapes, could result in complex effects on their life history.

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