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VISUAL AND GUSTATORY RESPONSES OF *SPODOPTERA FRUGIPERDA* (LEPIDOPTERA: NOCTUIDAE) LARVAE TO ARTIFICIAL FOOD DYES

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Abstract

Visual and gustatory responses of *Spodoptera frugiperda* Hübner (Lepidoptera: Noctuidae) larvae to artificial food dyes were studied. The objective was to determine the ability of these substances to attract and elicit feeding of insect larvae to assess their feasibility for improving the efficacy of ingested insecticides. The artificial food dyes lemon green, lemon yellow, orange red, chlorophyll and carmine red were used. Different blends were prepared with each dye to evaluate larval visual responses to color, larval preferences to color compared with a control, and larval gustatory responses. Only lemon green and lemon yellow dyes visually evoked the attraction of significant percentages of the larvae, but the other colorants did not elicit responses different from the control. Gustatory response tests indicated that larvae preferred to feed on gelatinized blends containing chlorophyll. The gelatinized blend containing lemon green dye was a strong gustatory repellent, and none of the other dyes evoked negative gustatory responses. Adding the artificial food dye lemon green to an insecticidal formulation could enhance insect attraction, while adding chlorophyll could enhance ingestion of the formulation. The study of sensory aspects of insect behavior can be useful for maximizing the efficiency of insecticidal formulations.

Key Words: visual stimuli, gustatory system, color, insecticidal formulation, attractants

Resumen

En este trabajo se estudiaron las respuestas visual y gustativa de larvas de *Spodoptera frugiperda* Hübner (Lepidoptera: Noctuidae) a colorantes artificiales para alimentos. El objetivo fue determinar la capacidad de estas sustancias para atraer y provocar la alimentación de las larvas, para determinar su factibilidad de uso en el mejoramiento de las formulaciones insecticidas. Se utilizaron los colorantes artificiales, verde limón, amarillo limón, naranja rojo, clorofila y rojo carmín. Se prepararon diferentes mezclas con cada colorante para evaluar la respuesta visual larvaria al color, preferencia larvaria al color y respuesta gustativa larval. Sólo los colorantes verde limón y amarillo limón ofrecieron un estímulo visual a un número significativo de larvas. Las mezclas que contenían verde limón y amarillo limón fueron preferidas visualmente, mientras que las que contenían el resto de los colorantes no provocaron ninguna respuesta diferente a la del control. Las pruebas de respuesta gustativa indicaron que las larvas prefirieron alimentarse de las mezclas gelatinizadas que contenían clorofila. Las mezclas gelatinizadas que contenían verde limón fueron las menos apetecibles. En este caso, la adición del colorante artificial verde limón a una formulación insecticida podría ser útil para la atracción de insectos, mientras que la clorofila puede ser utilizada para propósitos de ingestión. El estudio de los aspectos sensoriales de la conducta de los insectos puede ser útil para maximizar la eficiencia de formulaciones insecticidas.

Palabras Clave: estímulo visual, sistema gustativo, color, formulación insecticida, atrayente

Insecticidal formulations often contain a variety of adjuvants (Ignoffo et al. 1976), some of which are intended to lure the insect (Dethier et al. 1960; Rosas-García et al. 2004), while others are directed to protect and deliver the active ingredient. Although many insecticidal formulations have been used successfully to control many insect pests, some show limitations that may be remedied through addition of new compounds. Adjuvants include attractants, which are chemicals that cause insect to make source-oriented movements (Dethier et al. 1960). Some other adjuvants such as optical brighteners have been used for the same purpose. In addition, they may alter the chitinous peritrophic membrane by leaving a lesion, which may make an insect susceptible to infection (Shapiro & Robertson 1992). The use of dyes in insecticidal formulation has been limited primarily to protecting the active ingredient against solar radiation. However, dyes may offer other alternatives to improve insecticidal formulations if we consider that they are colored substances and can work as a visual cue. Considering that visual stimuli play an important role when insect needs to locate a suitable host plant (Hirota & Kato 2001), the visual behavior in insects becomes an important factor that can be used favorably for crop protection. The majority of insects possess highly developed visual systems. Particularly, holometabolous insect larvae possess visual organs called stemmata, which contain numerous photoreceptors that allow high light sensitivity (Gullan & Cranston 2008), and are spectrally differentiated in 3 types: UV, blue and green (Ichikawa & Tateda 1982). Photoreceptor cells found in members of the Lepidoptera, are able to sample a different portion of the spectrum and facilitate the discrimination of colors (Briscoe & Chittka 2001; Kelber et al. 2002; Buschbeck & Friedrich 2008). In addition, some lepidopterans also possess red receptors as observed in *Spodoptera exempta* (Pelzer & Langer 1990; Briscoe & Chittka 2001).

Lepidopteran larvae are among the most economically important foliage-eating pests (Gullan & Cranston 2008), and they are able to detect non-volatile compounds through the sense of taste (Schoonhoven & van Loon 2002; Amrein & Thorne 2005; Zhou et al. 2010). For this reason it is important to develop formulations visually attractive and palatable, because their success depends upon their ability to compete with host plant. The addition of different compounds may alter formulation flavor so it is important to determine if feeding is altered in some way. As there are no available data on how *Spodoptera* larvae respond to artificial food dye, the objective of this study was to determine if artificial food dyes are able to attract insects by their visual and gustatory responses in order to know the feasibility of using these harmless substances for the improvement of insecticidal formulations.

TABLE 1. ARTIFICIAL DIET FOR REARING *SPODOPTERA FRUGIPERDA*.

Ingredient	Amount (g/liter)
Soy flour	71.1
Wheat germ	31.7
Wesson salt	10.6
Sucrose	13.0
Sorbic acid	1.0
Methylparaben	1.6
Agar	14.0
Ascorbic acid	4.3
Acetic acid (25%)	12.0
Formalin (10%) *a*	4.4
Choline chloride *b*	7.3
Vanderzant vitamin mix	3.5

Procedure: Dissolve agar in boiling water, and then add all solid materials. Blend mixture in a blender for 10 min, and then add all liquid ingredients blending for 5 more min. After mixture is cooled slightly add vitamin mix and blend for 2 min, and immediately thereafter the diet mixture should be poured into 30-mL plastic cups.

[a]Prepare 37% formaldehyde and dilute to obtain a 10% solution.

[b]Dilute to a total volume of 1 liter by adding 150 mL of choline chloride.

MATERIALS AND METHODS

Insects

All *Spodoptera frugiperda* larvae used for this study were provided by the insect rearing facility of the Centro de Biotecnología Genómica-IPN. Artificial diet was prepared using the ingredients shown in Table 1. The rearing technique used was that described by Rosas-García (2002) with some modifications. Larvae of *S. frugiperda* were reared until pupation on 5 mL of the solidified diet contained in 30-mL plastic cups capped with cardboard lids. Pupae were collected and disinfected with 2% hypochlorite for 2 min and washed in running water, and then held for adult eclosion in emergence chambers, which consisted of 4-L plastic jars lined with paper towel and covered with a cheese cloth (30 cm × 30 cm) held in place with a rubber band. Adults laid eggs on the paper towel lining the inner cylindrical surface of the jar. Two 30 mL-plastic cups filled with a 15% sucrose solution were each plugged with a small cotton ball wick, and placed inside each plastic jar for feeding the adults. Eggs were collected daily by cutting out the area where the eggs were laid, and sometimes the eggs were lifted from the paper with a scalpel. Egg masses were placed into a hatching chamber, which consisted of a circular plastic base (3.0 cm diam × 0.7 cm deep) placed on the surface of the artificial diet contained in a 50 mL-plastic cup. The chambers were closed with a cardboard lid until egg hatch occurred. The insect cycle was carried out under controlled conditions (26 ± 1 °C, 14:10 h L:D and 65 ± 5% RH). Eggs were collected during 5 days, and the larvae were used for all tests.

Evaluation of Larval Visual Response to Color

Visual response tests were conducted to determine if color from artificial food dyes attracted larvae. For these tests the following powdered artificial food dyes were used, lemon green (green color), lemon yellow (yellow color), orange red (orange color), chlorophyll (dark green color), and carmine red (red color) (Medina Sabores y Fragancias S.A de C.V. Zapopan, Jal., Mexico). To provide a suitable matrix in which color can be seen, each artificial food dye was mixed with modified corn starch (Capsul™, Aranal, S.A. Monterrey, N. L., Mexico) at 1%. Subsequently, enough distilled water was added to the blend to form a paste. The paste was dehydrated in a forced-flow drying oven at 45 °C for 16 h. The dehydrated paste was finely ground in a mortar and kept in airtight containers for further use. Capsul™ does not attract larvae (Rosas-García et al. 2004), so it was used as an inert carrier to determine only visual response to color.

For the experiment, 50 mg of each powdered blend were placed as a mound on one side of a 5 cm Petri dish, and 10 neonate larvae were placed in the center of the Petri dish with a camel's hair brush. The Petri dish remained uncapped and the larval visual response was observed and recorded during 5 min. Each larva that approached to a distance of 1 mm or less from the mound was considered as having responded to its color.

A second experiment was conducted as previously described using new larvae but visual response was recorded during 10 min. All experiments were conducted with 5 replicates at room temperature under white light (Phillips, 40 watts, Slimline type) placed at a height of 1.6 m from the table level. Results were subjected to analyses of variance after arcsine transformation. Means were compared with Tukey's test at $P \leq 0.5$ (SPSS v. 15.0, SPSS Inc., Chicago, Illinois).

Two-Choice Tests for Larval Preference to Color

To determine if a particular color was preferred by larvae two-choice tests were conducted according to Bartelt et al. (1990) with some modifications. For these tests palatable powdered blends were prepared with ground dried corn at 5% (Rosas-García et al. 2009), one each of the artificial food dyes at 1% and sufficient modified corn starch (Capsul™) to obtain 50 g of the blend. Enough distilled water was added to each blend to form a paste. Blends were obtained with the same procedure followed for preparation of powdered blends mentioned above. In addition a control was prepared using the same ingredients without artificial food dye. In this case ground dried corn worked as a feeding stimulant to offer a palatable powdered blend in which color would be the only preference factor.

For each experiment, 50 mg of each of the palatable powdered blends was placed at opposite sides of a Petri dish forming 2 mounds. Ten 2-day old larvae were place in the center of the Petri dish, which remained uncapped. The larvae were allowed to move inside the dish for 10 min. Each larva found on the mound or near the mound (~1 mm) was removed with a camel's hair brush and recorded. All tests were conducted with 5 replicates and under the same conditions as previous experiments. Results obtained were statistically analyzed with the parametric paired t-test at $P \leq 0.05$, SPSS v. 16.

Two-Choice Test for Larval Gustatory Response

To determine if artificial food dyes elicit a gustatory response in larvae, gelatinized blends based on an artificial diet were prepared. Each artificial food dye at 1% was mixed with the freshly prepared artificial diet, and allowed to solidify. Ingredients for artificial diet are shown in Table 1. A two-choice test was also conducted to compare the gelatinized blend both with or without artificial dye. A 0.5 g quantity of each blend was placed at opposite sides of a 5 cm Petri dish. For each test only 1 third-instar larva was placed in the center of the dish leaving the larva to wander at room temperature during 18 h in complete darkness to avoid preference by color. The tests were conducted with 5 replicates. At the end of the experiment, the weight of consumed diet was determined by the difference between the original and remaining amounts of diet. Data obtained were compared by parametric paired t-test at $P \leq 0.05$, SPSS v. 16 to determine acceptance or rejection by larvae. Only the artificial food dye in the blend might give a different flavor that could be either palatable or unpalatable to larvae.

RESULTS

Results (Table 2) indicated that around 30% of *S. frugiperda* larvae responded visually to either lemon green or to lemon yellow dye to a significant extent when compared with rest of the dye treatments and the control during the 5-min-experiment ($F = 13.286$, df = 5,24,

TABLE 2. EVALUATION OF VISUAL RESPONSES OF *SPODOPTERA FRUGIPERDA* LARVAE TO EACH COLOR OF A DIET BLEND CONTAINING A POWDERED ARTIFICIAL FOOD DYE AND MODIFIED CORN STARCH AFTER EXPOSURE TIMES OF 5 AND 10 MIN.

	Mean percentage of responding larvae to color ± SE*	
Powdered blend⁺	5 min experiment	10 min experiment
SLG	33.08 ± 2.00 b	43.86 ± 4.30 b
SLY	30.55 ± 1.62 b	56.13 ± 4.02 b
SOR	14.02 ± 1.10 a	27.17 ± 4.09 a
SC	20.58 ± 2.63 a	17.85 ± 2.50 a
SCR	18.96 ± 2.18 a	21.39 ± 2.95 a
S	17.85 ± 2.50 a	23.53 ± 3.53 a

*$n = 5$, values in a column with the same letter are not significantly different. ANOVA and Tukey's test for mean comparison ($P \leq 0.5$). Percentage values were obtained from arcsine transformation from percentage of responding larvae.

Abbreviations: SLG-modified corn starch + lemon green; SLY-modified corn starch + lemon yellow; SOR-modified corn starch + orange red; SC-modified corn starch + chlorophyll; SCR-modified corn starch + carmine red; and S-modified corn starch.

$P \leq 0.001$) and in the 10 min experiment (F = 17.160, df = 5,24, $P \leq 0.001$).

Results from two-choice tests for larval color preferences involving artificial dyes mixed with modified corn starch and ground dried corn (Table 3) indicated that 50 ± 7.07% of larvae preferred to feed on a blend containing lemon green and 58 ± 3.74% preferred to feed on a blend containing lemon yellow over the control (SC), which did not contain any dye. Larval color preference for chlorophyll, carmine red or orange red did not differ significantly from the control.

Results from gustatory response (Table 4) indicated that larvae strongly preferred to feed on gelatinized blends containing chlorophyll compared to no chlorophyll (control). Because the experiment was conducted in complete darkness, color preferences did not play a role. The only possible effect of the dyes could be that on palatability. On the other gelatinized blend containing lemon green evoked a repellant (negative) gustatory response. None of the gelatinized blends containing the rest of the colorants evoked a gustatory response significantly different from the control.

Discussion

Results from this study indicate that some artificial food dyes such as lemon green and lemon yellow evoked visual responses of *S. frugiperda* larvae, and that such responses to color occurred rapidly and continuously as progressively more larvae approached near to the colored blends. Responses towards green and yellow colors were expected in this study as they are in agreement with the possession of UV, blue, and green receptors by almost all insects (Briscoe & Chittka 2001).

Responses to color tests were intended to determine if - in some way - color stimulated a preference for a visually palatable blend. Palatable blends containing lemon green and lemon yellow dyes were preferred visually by larvae, while blends containing orange red, chlorophyll and carmine red dyes did not elicit any visual response different from the control. Because the colored dyes were blended into the food, it is possible that larvae were attracted simultaneously by food and color. However lemon green and to a greater extent lemon yellow evoked larval visual responses, whereas the other tested artificial food dye seemed not to evoke attraction by the larvae. After determining that certain colors play a role in larval attraction, it was important to determine if artificial food dyes blended into the diets also evoked larval gustatory responses. Thus we found that only chlorophyll evoked a significantly positive gustatory attraction and only lemon green evoked strong repellency (significantly negative gustatory response).

Although insects can associate colors with food (Salloum et al. 2011), our only interest was to determine if these compounds (dyes) increased feeding. Our finding that larvae preferred to feed on blends containing chlorophyll was not surprising, because it is a compound normally consumed by larvae.

The above results should be considered in the development of insecticidal formulations. Thus the addition of artificial food dyes lemon green and lemon yellow in insecticidal formulations could enhance insect attraction, while chlorophyll could enhance ingestion.

Insect behavior is an important consideration in the development of nonchemical techniques. Aphids and many other insects prefer yellow over green color because yellow has a high reflectance in the range where the green receptor is sensitive (Kelber 2001; Döring & Chittka 2007). However this is not a true color preference, because yellow is preferred as a result of its brightness effect. These kinds of responses are relevant because they can be used advantageously for optimization of insecticide products.

Unfortunately there are few studies related to visual stimuli behavior in *Spodoptera*, however, our findings agree with those of Singh & Saxena (2004) in which *S. litura* larvae responded preferably to

TABLE 3. VISUAL RESPONSES OF *SPODOPTERA FRUGIPERDA* LARVAE IN TWO-CHOICE TESTS COMPARING RESPONSES TO THE DIET BLEND EITHER WITH OR WITHOUT AN ARTIFICIAL DYE.

Treatment	Mean percentage of responding larva ± SE	t	2-tailed *P*
SC vs SCLG	12.00 ± 5.83 *vs* 50.00 ± 7.07	-3.413	0.027*
SC vs SCLY	20.00 ± 3.16 *vs* 58.00 ± 3.74	-7.757	0.001*
SC vs SCOR	18.00 ± 4.89 *vs* 26.00 ± 5.09	-0.930	0.405
SC vs SCC	34.00 ± 7.48 *vs* 30.00 ± 7.07	0.286	0.789
SC vs SCCR	22.00 ± 5.83 *vs* 18.00 ± 3.74	-0.667	0.541

A two-choice bioassay was conducted for each pair of treatments. Paired t-test, $P \leq 0.05$.

*Significantly different.

Abbreviations: SCLG-modified corn starch + ground dried corn + lemon green; SCLY-modified corn starch + ground dried corn + lemon yellow; SCOR-modified corn starch + ground dried corn + orange red; SCC-modified corn starch + ground dried corn + chlorophyll; SCCR- modified corn starch + ground dried corn + carmine red; and SC-modified corn starch + ground dried corn.

TABLE 4. GUSTATORY RESPONSES OF *SPODOPTERA FRUGIPERDA* LARVAE TO THE PRESENCE OR ABSENCE OF ARTIFICIAL FOOD DYES BLENDED INTO A GELATINIZED ARTIFICIAL DIET IN TWO-CHOICE TESTS.

Treatment	Ingested blend mean values (g)	t	2-tailed *P*
DLG vs D	0.133 vs 0.248	-2.483	0.038*
DYG vs D	0.201 vs 0.171	1.271	0.239
DOR vs D	0.239 vs 0.136	1.854	0.101
DC vs D	0.245 vs 0.143	4.009	0.004*
DCR vs D	0.202 vs 0.162	1.751	0.118

A two-choice bioassay was conducted for each pair of treatments. Paired t-test, $P \leq 0.05$. *Significantly different.
Abbreviations: DLG-artificial diet + lemon green; DLY-artificial diet + lemon yellow; DOR-artificial diet + orange red; DC-artificial diet + chlorophyll; DCR-artificial diet + carmine red; and D-artificial diet.

green and yellow colors. We did not find an important response to other colors such as carmine red or orange red, possibly because *S. frugiperda* might not possesses red receptors as those mentioned in *S. exempta* (Briscoe & Chittka 2001). Biologically-based insect pest suppression programs should include the use of improved formulations that take advantage of sensory responses of insects to maximize the protection agriculturally important crops.

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