ECOLOGY, BEHAVIOR AND BIONOMICS

Effect of Stereoisomers of the Main Component of the Sex Pheromone of *Euschistus heros* (F.) (Hemiptera: Pentatomidae) in the Attractiveness of Females

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Efeito dos Estereoisômeros do Principal Componente do Feromônio Sexual de *Euschistus heros* (F.) na Atratividade de Fêmeas

RESUMO - A atratividade de fêmeas de *Euschistus heros* (F.) (Hemiptera: Pentatomidae) aos oito estereoisômeros do 2,6,10-trimetiltridecanoato de metila, principal componente do feromônio sexual produzido pelos machos da espécie, foi estudada em olfatômetro de dupla escolha. Os bioensaios demonstraram que o estereoisômero (2R, 6R, 10S) foi necessário para a atratividade das fêmeas até a fonte de odor, destacando-se em relação aos outros. Apesar de exercer atratividade significativa para as fêmeas, a mistura de estereoisômeros quando comparada ao (2R, 6R, 10S) apresentou menor atratividade.

PALAVRAS-CHAVE: Insecta, 2,6,10-trimetiltridecanoato de metila, semioquímicos, mistura de estereoisômeros.

ABSTRACT - The attractiveness of *Euschistus heros* (F.) (Hemiptera: Pentatomidae) females to the eight stereoisomers of the methyl 2,6,10-trimethyltridecanoate, major component of the sex pheromone produced by the males of this species, was studied in a double choice olfactometer. Bioassays showed that the (2R, 6R, 10S) stereoisomer was necessary for the attractiveness of females, presenting better results in relation to the others. The stereoisomeric mixture was shown to present significative attractiveness to females, however, when compared to the (2R, 6R, 10S) stereoisomer, it showed lower attractiveness.

KEY WORDS: Insecta, methyl 2,6,10-trimethyltridecanoate, semiochemicals, stereoisomeric mixture.

Among the species of *Euschistus* spread around the world, some frequently coexist with other pentatomids such as *Acrosternum hilare* (Say) and *Nezara viridula* (L.), constituting a harmful complex to the soybean crop (Turnipseed 1976, Sediyama *et al.*1993). *Euschistus heros* (F.), has been considered the most serious pest of the Brazilian soybean in the last decades (Panizzi & Rossi 1991). The identification, as well as the attractiveness of semiochemicals present in several *Euschistus* species was reported by Aldrich *et al.* (1991). Also, studies of *E. heros* behavior were initiated with the elucidation of its system of chemical communication based on the Neartic species, *Euschistus obscurus* (Palisot) (Borges & Aldrich 1994, Aldrich *et al.* 1994). Biological activity of *E. heros* females to the stereoisomeric mixture of methyl 2,6,10-trimethyltridecanoate, the major component of the sex pheromone produced by the males of the species was evaluated (Borges *et al.* 1998a). Recently, bioassays performed by Borges *et al.* (1998a) demonstrated the presence of a circadian rhythm that could lead the insects to mate in certain periods of the day.

An effective synthetic attractant for the species of *Euschistus* would be an useful tool for population monitoring of such a pest (Aldrich *et al.* 1991). Furthermore, it could also be used to concentrate the pentatomids in early maturing trap crops, where they could be economically destroyed by limited applications of insecticides (McPherson & Newsom 1984).

The objective of this study was to infer the stereochemistry of the natural methyl 2,6,10- trimethyltridecanoate, from *E. heros.* Bioassay studies were conducted to verify which of the eight stereoisomers synthesized by Mori & Murata (1994a, b) would present biological activity. Also, the study supports the olfactometry technique as an aid to the identification of semiochemicals for such species.

Material and Methods

Insects. Adults of *E. heros* were reared on green bean pods (*Phaseolus vulgaris*), soybeans (*Glycine max*) and peanuts (*Arachis hypogaea*) at 24±0.5°C, 70±10% RH and 14 h of photophase. To prevent olfactory interactions between the sexes, after emergence, adults were separated by sex. Males were separated from females until sexual maturity, a period of about 11 days (Costa *et al.* 1998).

Semiochemical Extracts. The eight synthetic stereoisomers of methyl 2,6,10-trimethyltridecanoate (Mori & Murata 1994a, b) were kindly provided by Prof. Kenji Mori from Tokyo University (Chemistry Department). In preliminary bioassays, different concentrations of the stereoisomerc mixture were tested in an arena olfactometer. The monitoring of the attractiveness to the compounds was accomplished in a double choice olfactometer.

Arena Olfactometer. This olfactometer was modified from Borges *et al.* (1998a) in order to choose the optimal concentration of the stereoisomeric mixture to be used in the bioassays. The device consisted of an acrylic box of 24 x 24 x 8 cm. The scent sources were placed in card paper boxes (3.3 x 3.3 x 1.5 cm) similar to the Folding Fumigant Boxes (BioQuip Products - Gardena, CA, USA), adjusted at the opposite vertexes of the acrylic box, without a forced air flow. In the center of the arena, an acrylic cap (4.4 x 4.5 x 2.5 cm) was used as an insect release chamber. The base of a Petri dish was used as a lid for the release chamber, avoiding insect evasion. A 3 mm mesh screen also covered the 17 x 17 cm olfactometer opening, preventing the saturation of the internal atmosphere. A metallic mesh (0.5 mm) covered the sides of ca. 5 cm^2 of the card paper boxes where the scent sources were located. The olfactometer was placed on a horizontal surface in a room with temperature and humidity similar to the one where the insects were reared. An exhaustion duct continually renewed the air of that room.

Bioassay. A range of concentrations were

tested for the attractiveness of *E. heros* females to the stereoisomeric mixture of the methyl 2,6,10–trimethyltridecanoate. Four arena olfactometers, operating simultaneously, but independently from one to another, were used in each replicate of the bioassay. Ten virgin females (sexually mature, 12 days old) were released in the center of the olfactormeter for each replicate. Each arena received one of the following concentrations of the stereoisomeric mixture (treatments): 2.0; 0.2; 0.02; 0.002 µg/µl. The stereoisomeric mixture as well as the *n*-hexane used as control was applied to rubber septa with 5 ml microcap (Drumond). The septa were then set inside the card paper boxes designated for treatment and control.

Effects of the different concentrations on the behavioral responses of females were determined by recording the number of females at the top of the boxes, or at a distance ≤ 2 cm, in 1 h period. Each replicate was carried out in one position, rotating the boxes in a clockwise manner, in attempt to minimize any light or environmental condition influences. A total of 29 replicates were accomplished at 24±0.5°C and 70±10% RH. Following each bioassay, the whole system was washed and the card paper boxes were left in a stove at 80°C during 24 h to remove any possible volatile substances.

The differences among concentrations were tested by ANOVA ($P \leq 0.05$) (SYSTAT, Systat Inc.; Wilkinson 1990). Due to the great variation among female responses, the χ^2 test was used to compare their attractiveness to the treatments and control.

Double Choice Olfactometer. An olfactometer modified from Borges (1995), was used to test the attractiveness of the stereoisomers of methyl 2,6,10-trimethyltridecanoate. The olfactometer consisted of three rectangular acrylic boxes, two of them measuring 20 x 10 x 8 cm and the third, release arena, 20 x 10 x 16 cm. The release arena was connected to an exhaust pipe on one of its sides. The other side was connected by glass tubes (29.5 x 3.5 cm) to the other two boxes: treatment and control arenas. These boxes were placed side by side, in front of a turbocalefactor model Punk Tal of simple phase, controlled by a variable voltage transformer, Variac. Activated charcoal filters were attached to the ventilation system. The airflow was also controlled to form a linear plume. It was conducted to the treatment and control arenas through a scentless plastic tube connected to the turbocalefactor. Throughout the mentioned arenas, the airflow led the scent plume to the insects release arena and the glass tubes, leaving the olfactometer throughout the exhaustion system, controlled by a potentiometer. The insects were allowed to reach the control and treatment arenas by two steel funnels. Once inside the arenas, they were impeded to leave by the same funnel.

The calibration of the olfactometer was accomplished using the "smoke test" (Kellog & Wright 1962). A source of smoke is essential to demarcate the average time of the pheromone plume, making the visualization of airflow through all the areas of the olfactometer possible (Baker & Linn 1984).

Bioassay. The eight stereoisomers (Fig.1), derived from the methyl 2,6,10–trimethyltridecanoate, were tested separately using the concentration of 0.2 µg/µl. Each stereoisomer was used in a first instance as an individual treatment, totaling 18 replicates, divided in two daily bioassays. Following this procedure, bioassays testing the interactions among the stereoisomers that presented significant attractiveness were accomplished, observing effects as synergism, inhibition, or activation of one component by another. Each stereoisomer was combined following proportions of 50% or 100%. The number of 18 replicates were maintained, at the same temperature and humidity conditions as those of the initial tests.

Methyl (2R, 6R, 10S) - trimethyltridecanoate was the most attractive stereoisomer of all. It was tested against the stereoisomeric mixture, considered initially to be the source of greater attractiveness to the *E. heros* females. The numbers of replicates

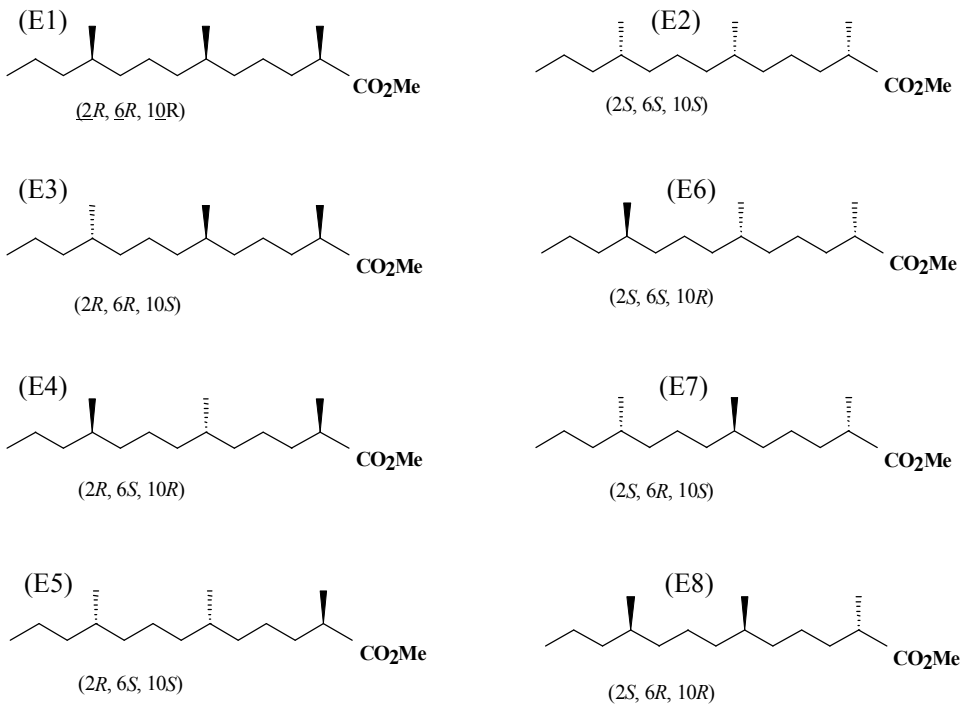

Figure 1. Stereoisomers of methyl 2,6,10-trimethyltridecanoate, a major component of the sex pheromone of *E. heros* (Mori & Murata, 1994 b).

as well as temperature and humidity conditions were kept as before.

The bioassay procedure used by Borges (1995) with some modifications was followed: the bioassays were allowed to run for 30 min for all computed data, between 15:00 and 19:00 h, when the females demonstrated significant attraction to the sex pheromone. A total of 10 sexually mature virgin females, of standardized ages, were placed into the release arena. Prior to testing, the insects were allowed to acclimatize for a period of 30 min in the release arena before the attachment of the other olfactometer sections. Treatment and the control were applied to a rubber septa with 5 µl microcap (Drumond), positioned inside the respective treatment and control arenas. The solvents were allowed to evaporate for 20 sec. The insects were only used once for each replicate in the experiment.

Only the long distance attractiveness behavior described by Borges *et al.* (1987) was considered, to monitor the biological activity of the synthetic complexes, i.e., a single stereoisomer or a combination of stereoisomers should be active enough to make the insects move towards the vicinity of the scent source. Five ml of *n*-hexane was used as control in all bioassays.

ANOVA (SYSTAT, Systat Inc.; Wilkinson 1990) was used to analyze the number of females responding to treatments by comparing the averages using the Tukey Test ($P \leq 0.05$).

Results

There was no significant difference

(F=1.019; P=0.387) among the different concentrations of the stereoisomeric mixture of methyl 2,6,10-trimethyltridecanoate (Fig. 2). However these treatments attracted, in terms was towards the samples containing the (2R, 6R, 10S) stereoisomer (F=10.639; P<0.001, Tukey Test, P≤0.05) (Table 2). Result demonstrated that the absence of that stereoisomer

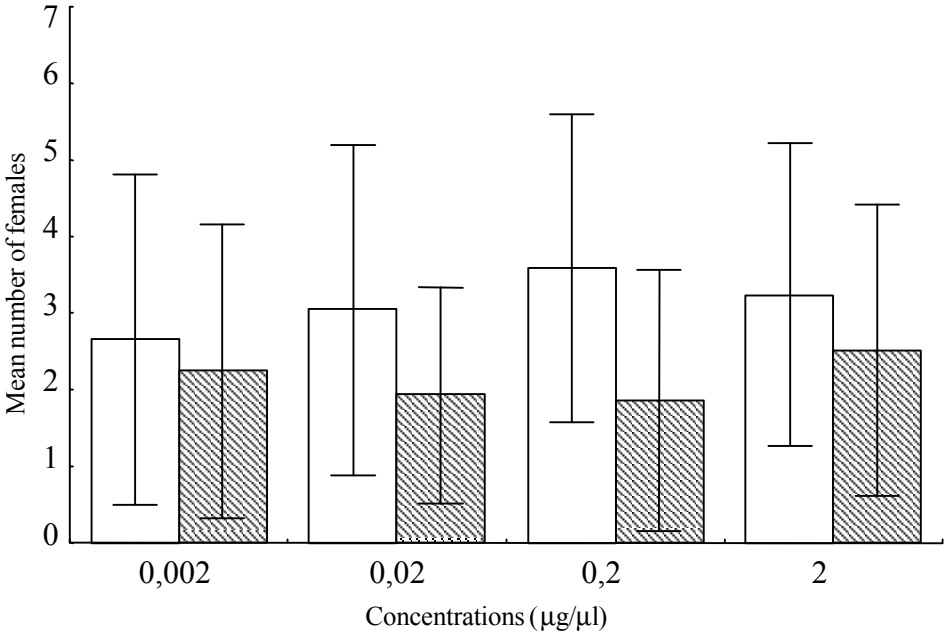

Figure 2. Mean number of *E. heros* female responding to treatments (white barrs) and control (bolded barrs) of different concentrations of the stereoisomeric mixture of methyl 2,6,10-trimethyltridecanoate (n=290, 10 females/replicate).

of numbers, more insects than the solvent used as control (Friedman's Test χ^2 =14.156; P<0.001). The 0.2 µg/µl was chosen for the bioassays with each one of the eight stereoisomers. There was a significant difference among the average responses to these compounds (F=50.429; P<0.01) (Fig. 3). The (2R, 6R, 10S), (2S, 6S, 10R) and (2S, 6R, 10S) stereoisomers attracted the females to their vicinities. On the other hand, the (2R, 6S, 10S) stereoisomer evoked a totally contrary effect on the insects (Table 1).

By testing the ratios of 1:1:0, 1:0:1 and 0:1:1 of the cited stereoisomers, it was concluded that the greater female attractiveness caused a notable decrease in the female's response to the treatments (Fig. 4). The combination which used 1:1:1 ratio of (2R, 6R, 10S), (2S, 6S, 10R) and (2S, 6R, 10S) stereoisomers did not increase responses (Fiedman's Test χ^2=5; P=0.480). When compared to others, the 1:1:1 ratio of stereoisomers still presented a low attractiveness to the females (Fig. 4).

The attracted females reached the scent source through directed movements inside the double choice olfactometer tube, remaining close to or above the source of stimulus, located at the end of the treatment arena. The insects that moved toward the control presented randomize movements or simply stood

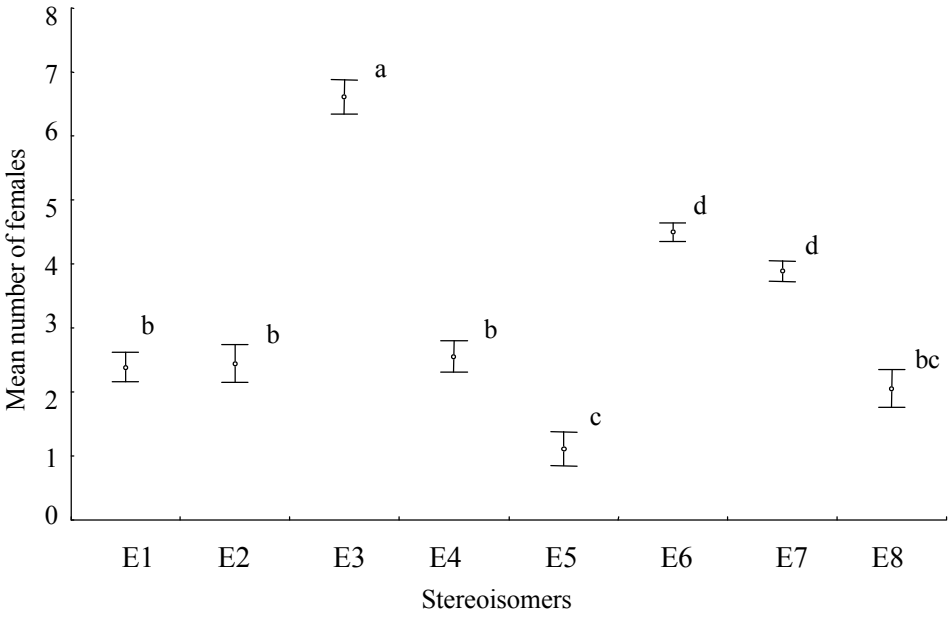

Figure 3. Mean number of *E. heros* female responding to the stereoisomers of methyl 2,6,10-trimethyltridecanoate (n=180, 10 females/replicate). Values followed by the same letter are not significantly different (Tukey's Test (Friedman's, P≤0.05)). The stereoisomers are represented by the symbols E1 to E8 according to Fig. 1.

still for the entire period of observation. On some occasions, the females left the control tube, following the direction of the release chamber, elevating the antennae above their heads, positioning them in a "V" form, as observed for *N. viridula* (Borges *et al.* 1987). That procedure was followed until they relocated the scent plume.

The results showed that the (2R, 6R, 10S) stereoisomer, as well as the stereoisomeric mixture of methyl 2,6,10-trimethyltridecanoate, attracted *E. heros* females. However, when both cases were compared, the (2R, 6R, 10S) had greater activity (Friedman's Test χ^2 =10; P< 0.002).

Discussion

The multicomponent nature and the absolute configuration of pheromones have contributed to their specificity (Vilela & Della Lúcia 1987). Nevertheless, many pheromones are isolated in low quantities for determination of their absolute stereochemistry. An alternative is to infer the correct structure of the natural product from synthetic isomers. In this case, the major difficulty is to determine the pheromone components of such products (Rockstein 1978).

The attractiveness of a combination of (2R, 6R, 10S), (2S, 6S, 10R) and (2S, 6R, 10S) stereoisomers in the ratio of 1:1:1 was less than that for each stereoisomers tested separately. This observation suggests that there is no additive effect or synergism among the components, since when tested individually, they attracted a larger number of *E. heros* females.

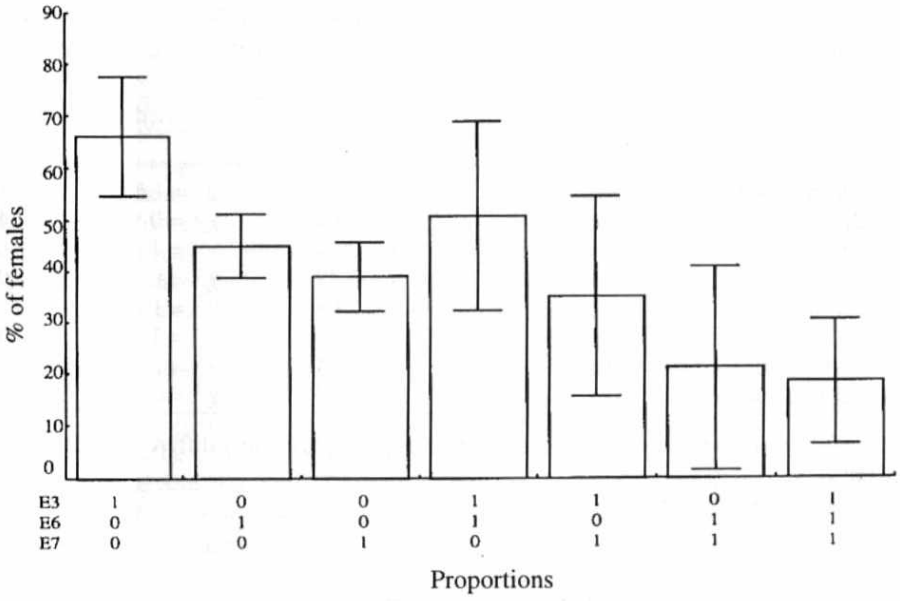

Figure 4. Percentage of *E. heros* female responding to the most attractive stereoisomers of methyl 2,6,10-trimethyltridecanoate, tested separately and combined in different proportions (n=180, 10 females/replicate). E3 = (2*R*, 6*R*, 10*S*); E6 = (2*S*, 6*S*, 10*R*); E7 = (2*S*, 6*R*, 10*S*) stereoisomers, respectively.

The evidence that the (2R, 6R, 10S) was absolutely essential to pheromonal activity was evident when tests with different combinations of the three most attractive stereoisomers were initiated. The absence of the (2R, 6R, 10S) stereoisomer in the scent sources presented to the *E. heros* females always caused a decrease in the average response (Table 1).

Although the stereoisomeric mixture of methyl 2,6,10-trimethyltridecanoate evoked significant attraction to the females (Borges *et al.* 1998a, 1998b), it was less attractive than the (2R, 6R, 10S) stereoisomer alone.

The use of the correct stereoisomer may be very important when regarding biological systems, since the presence of a lower quantity of another stereoisomer may evoke an acute inhibitory effect. The existence of other stereoisomers may disguise the behavioral responses of the *E. heros* females to the synthetic pheromone, diminishing its attractiveness, as appears to occur with the (2R, 6S, 10S) stereoisomer. However, the reason of why this happens is unknown.

In this study, *E. heros* was attracted predominantly to the (2R, 6R, 10S) stereoisomer of methyl 2,6,10-trimethyltridecanoate. Possibly, it could be used as a long distance attraction mechanism. Even if the functions of the minor components of the sex pheromone of those insects remain unknown (Adrich *et al.* 1991), these components might exert some type of short range attractiveness coordinating both sexes, once the insects are near, and then, trigger the mating behavior. An "oriented approximation", would mark the transition between the sequence of long range attraction and courtship (Borges *et al.* 1987). Once the short-range behavior is initiated, it would be incremented by visual stimuli (Matthews & Matthews 1978) as well as

Table 1. Mean number of female *E. heros* responding to the eight stereoisomers presented in the mixture of methyl 2,6,10-trimethyltridecanoate (n=180, 10 females/replicate).

Stereoisomers	Treatments[1] (X ± SD)	Control (X ± SD)	Friedman's Test[2]	
E1 - (2*R*, 6*R*, 10*R*)	2.4 ± 0.95 b	2.8 ± 1.80	$\chi^2 = 1.667$	P = 0.197
E2 - (2*S*, 6*S*, 10*S*)	2.4 ± 1.21 b	2.9 ± 1.68	$\chi^2 = 0.529$	P = 0.467
E3 - (2*R*, 6*R*, 10*S*)	6.6 ± 1.11 a	0.7 ± 0.74	$\chi^2 = 18$	P < 0.001
E4 - (2*R*, 6*S*, 10*R*)	2.5 ± 1.01 b	2.9 ± 1.49	$\chi^2 = 0$	P = 1.000
E5 - (2*R*, 6*S*, 10*S*)	1.1 ± 1.10 c	4.5 ± 2.08	$\chi^2 = 16$	P < 0.001
E6 - (2*S*, 6*S*, 10*R*)	4.5 ± 0.60 d	1.7 ± 1.10	$\chi^2 = 18$	P < 0.001
E7 - (2*S*, 6*R*, 10*S*)	3.9 ± 0.66 d	1.9 ± 0.84	$\chi^2 = 18$	P < 0.001
E8 - (2*S*, 6*R*, 10*R*)	2.0 ± 1.22 bc	1.7 ± 1.29	$\chi^2 = 0.692$	P = 0.405

[1] Values followed by the same letter in a column are not significantly different (Tukey's Test, $P \leq 0.05$);
[2] Friedman's Test ($P \leq 0.005$).

acoustics (Harris *et al.* 1982), which also play an important role as mediators of sexual communication. That could explain the absence of complete mating behavior concern-

stroyed by a limited chemical and/or biological insecticide application. Aldrich *et al.* (1997) have been utilizing synthetic pheromones successfully to increment the bio-

Table 2. Mean number of female *E. heros* attracted by different proportions of the most attractive stereoisomers[1] presented in the stereoisomeric mixture of the methyl 2,6,10–trimethyltridecanoate (n=180, 10 females/replicate).

Proportions (E3:E6:E7)[1]	Females (SD)
1: 1: 0	5.1 (1.78) a
1: 0 : 1	3.5 (1.90) b
0: 1: 1	2.1 (1.91) b

[1]E3 = (2*R*, 6*R*, 10*S*); E6 = (2*S*, 6*S*, 10*R*); E7 = (2*S*, 6*R*, 10*S*);
Values followed by the same letter are not significantly different (Tukey's Test, $P \leq 0.05$).

ing the *E. heros* females when attracted to methyl (2R, 6R, 10S) - trimethyltridecanoate.

Methyl (2R, 6R, 10S) - trimethyltridecanoate may be useful for alternative tactics to control *E. heros.* For example, to concentrate them in areas of early maturing trap crops where they could be economically de-

logical control with *Podisus* and *Supputius*'s predators. In that case, devices containing pheromones attract the predators to a specific area where they are able to control certain pests.

More research needs to be conducted to determine, for instance, if high levels of me-

thyl (2R, 6R, 10S) - trimethyltridecanoate released in crops could disrupt the mating of *E. heros*. Once decoded the communication system used by these insects, this stereoisomer may be used in different ways. Initially, attracting females to traps or devices strategically placed in soybean fields, may be an effective way to monitor for the presence of the pest. And finally through continuous liberation aiming to effectively interrupt the mating process of those pests.

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