

Short communication. Pheromone inhibitors for *Pandemis pyrusana* males (Lepidoptera: Tortricidae)

T. Curkovic^{1*} and J. F. Brunner²

¹ Departamento de Sanidad Vegetal. Universidad de Chile. Casilla 1004. Santiago. Chile

² Tree Fruit Research and Extension Center. Washington State University. Wenatchee, WA, 98801. USA

Abstract

A grease matrix was loaded with pheromone components for *Pandemis pyrusana* and *Choristoneura rosaceana* (Lepidoptera: Tortricidae), two synchronic and sympatric species affecting apple orchards in Washington, USA. The chemicals used were a 5-blend of cis-11-tetradecenyl-acetate (Z11-14Ac), trans-11-tetradecenyl-acetate, cis-11-tetradecenyl-1-ol, cis-11-tetradecanal, and cis-9-tetradecenyl-acetate (Z9-14Ac) (90.05:1.89:1.42:0.95:5.69 ratio), or a 2-blend of Z11-14Ac and Z9-14Ac (94:6 ratio). Experiments to test each blend separately in a wind tunnel (at 0.16% and 16% pheromone), or in field trials (1.6% and 16%), indicated that significantly more *P. pyrusana* males responded to the higher concentration within any blend, and that a source containing the 5-pheromone blend caused significant reduction in attractiveness to this species. Source contact was significantly reduced within the *P. pyrusana* male behavioral sequence. This is the first report for *P. pyrusana* pheromone inhibitor, but further research is needed to identify whether one or more specific components elicit this response. The inhibitory effect observed probably helps to avoid interspecific matings in nature, and might be useful to develop some strategies against this pest, but it impedes to developing an attracticide formulation targeting simultaneously *P. pyrusana* and any other sympatric and synchronic species using these chemicals (as *C. rosaceana*) within their pheromone blends.

Additional key words: attracticides, *Choristoneura rosaceana*, pest control, pheromone components.

Resumen

Comunicación corta. Feromonas inhibitoras para machos de *Pandemis pyrusana* (Lepidoptera: Tortricidae)

Se utilizaron mezclas de compuestos químicos que forman parte de una feromona descrita para *Pandemis pyrusana* y *Choristoneura rosaceana* (Lepidoptera: Tortricidae), dos plagas sincrónicas y simpátricas que atacan huertos de manzanos en Washington, EEUU. Estas mezclas fueron de 5 componentes: cis-11-tetradecenil-acetato (Z11-14Ac), trans-11-tetradecenil-acetato, cis-11-tetradecenil-1-ol, cis-11-tetradecanal y cis-9-tetradecenil-acetato (Z9-14Ac), en proporción de 90.05:1.89:1.42:0.95:5.69, ó de 2 componentes: Z11-14Ac y Z9-14Ac, en proporción 94:6, y fueron incorporadas a una matriz grasosa, hasta lograr el 16%, 1,60% ó 0,16% de cada mezcla. Se condujeron ensayos en un túnel de viento con cada mezcla en forma independiente (al 0,16% y 16%), y otro en el campo (al 1,6% y 16%). Estos estudios mostraron que significativamente más machos respondieron a las concentraciones más altas para ambas mezclas, y que la mezcla de cinco componentes redujo significativamente la atracción de machos de *P. pyrusana* en laboratorio y campo. El contacto con la fuente, dentro de la secuencia de comportamiento, se redujo significativamente. Este es el primer reporte de inhibidores de feromona para *P. pyrusana*, pero se requiere investigar específicamente si uno o más de estos componentes indujeron estas respuestas. El efecto observado puede ayudar a evitar cópulas inter-específicas en la naturaleza y podría ser útil en el desarrollo de estrategias de control contra esta especie, pero es un impedimento para el desarrollo de attracticidas para el control simultáneo de *P. pyrusana* y otra(s) especie(s) simpátricas y sincrónicas que usen estos compuestos químicos (como *C. rosaceana*) como componentes de su feromona sexual.

Palabras clave adicionales: attracticidas, componentes de feromona, control de plagas, *Choristoneura rosaceana*.

Sex pheromone loaded attracticides have been reported for several Tortricidae (Curkovic and Brunner,

2003; Evenden and McClaughlin, 2005). This pest control technique relies on point source applications (Krupke *et al.*, 2002), and their efficacy depends on the attractancy power, and the feasibility to induce contact of males to the sources (Curkovic and Brunner, 2005). The

* Corresponding author: tcurkovi@uchile.cl
Received: 21-06-06; Accepted: 24-05-07.

possibility of controlling simultaneously two or more pests by mixing their respective pheromones into an attracticide formulation might be economical, and serve to manage several target species at the same time. However, their suitability depends on possible interactions between pheromone components of the target species, e.g. modification of ideal ratios or the presence of inhibitors within the formulation. Several tortricid species use the same pheromone components to attract conspecific males, e.g. *cis*-11-tetradecenyl-acetate is the main component present in closely similar ratio for *Pandemis pyrusana* Kearfott and *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae) (Arn, 1991), two synchronic and sympatric apple (*Malus domestica* Borkh.) pests in Washington State, USA (Curkovic, 2004). Some compounds added to a species' sex pheromone blend can cause cessation of attraction in conspecific male. In general, compounds leading to this type of response have been called pheromone inhibitors (Witzgall and Priesner, 1991). However, the word antagonist is also used (Renou and Guerrero, 2000) when these components belong to the sex pheromone blends of sympatric species (Fadamiro *et al.*, 1999), playing a role in preventing costly reproductive mistakes (Gemeno *et al.*, 2006). The objective of this research was to test responses from *P. pyrusana* males to sources containing different concentrations of both, a blend including all pheromone components for *P. pyrusana* and *C. rosaceana* together, *vs.* the *P. pyrusana* blend alone, in order to evaluate the feasibility to developing an attracticide targeting sympatric species that share pheromone components.

Pandemis pyrusana insects were obtained from colonies maintained at the Washington State University Tree Fruit Research and Extension Center (TFREC-WSU), Wenatchee, WA, USA. Colonies were maintained in growth chambers at $23 \pm 2^\circ\text{C}$, 40-50% HR, and 16:8 h photoperiod. Insect management was as described by Curkovic (2004).

Chemicals components used for pheromone blends were provided by Bedoukian Research Inc. (Danbury, CT); all were at least 95% pure. Pheromones used were: *cis*-9-tetradecenyl-acetate (Z9-14Ac), *cis*-11-tetradecanal (Z11-14Al), *cis*-11-tetradecenyl-1-ol (Z11-14OH), *cis*-11-tetradecenyl-acetate (Z11-14Ac), and *trans*-11-tetradecenyl-acetate (E11-14Ac). The matrix used in experiments was a grease commercial formulation (Last Call™)¹ that includes an ultraviolet stabilizer

comprising over 70%, with the remainder of the inert ingredients, including a thickener and a sticker; no insecticide was added into it.

One batch was prepared using the reported ratio by Roelofs *et al.* (1977) for *P. pyrusana* (94 Z11-14Ac: 6 Z9-14Ac, 2-pheromone blend). The components were added directly to the matrix to prepare a 16% (w:w) mixture, then stirred while in a warm-bath, poured into a syringe, and stored at 0°C . Besides, another batch (5-pheromone blend) containing 16% 90.05 Z11-14Ac:1.89 E11-14Ac:1.42 Z11-14OH:0.95 Z11-14Al:5.69 Z9-14Ac was prepared to obtain a single source with both, *P. pyrusana* and *C. rosaceana* sex pheromone components, satisfying the reported ratio for each species (the *C. rosaceana* blend, 96.5 Z11-14Ac:2 E11-14Ac:1.5 Z11-14OH:1 Z11-14Al, was reported by Vakenti *et al.*, 1988).

A wind tunnel, similar to that described by Cardé and Hagaman (1979), was built at the TFREC-WSU in an environmentally controlled room set at $25 \pm 2^\circ\text{C}$. The airspeed was ca. 45 cm s^{-1} in the middle of the chamber. Relative humidity was $55 \pm 5\%$ while light intensity was 2 lux during assays.

Males management, handling, and observation was described by Curkovic (2004). Trials were run in the first 2-4 h of the scotophase, which correspond to the sexual activity period for *P. pyrusana* (Knight and Turner, 1998). One treatment (a particular concentration for a given blend) was run per night. Male responses were categorized as staying in, taking off from the platform, or making source contact with the source cage.

Attracticide droplets were placed inside cages (hair rollers; 6 cm length, 1.5 diameter, L&N Sales and Marketing, PA), on a source platform held by a ring stand at approximately 10 cm from the upwind end, in the middle of the wind tunnel plume. In field trials, one drop ($50 \pm 0.1 \text{ mg}$) of the attracticide formulation was placed on a piece of aluminum foil in a hair roller that was pinned inside a Delta trap, and then placed on an apple tree (1.5-2 m) in an orchard at Wenatchee, central Washington. Traps ($n = 4$) were separated at least 30 m, then checked, serviced, and rotated clockwise after each examination to minimize the impact of location on captures. The orchard was not sprayed with conventional insecticides during the trial period.

A χ^2 test and an analogous of the Tukey test were used to identify multiple differences between propor-

¹ Provided by Phillip Kirsch, IPM Inc. Technologies, Portland, OR, USA.

Table 1. Behavioral responses of *P. pyrusana* males (#) exposed to attracticide sources containing different pheromone blends and concentrations in a wind tunnel (n = 30 males/treatment), χ^2 and Tukey test (P = 0.05)

Pheromone blend at source	% pheromone (w/w) at source	SP ¹	TO ²	CS ³
5-pheromone blend ⁴	0.16	15 a	15 a	0 c
2-pheromone blend ⁵	0.16	16 a	12 a	2 bc
5-pheromone blend	16.00	10 a	17 a	3 b
2-pheromone blend	16.00	10 a	11 a	9 a

¹ SP: staying on platform. ² TO: taking off. ³ CS: contacting source. ⁴ Ratio of components in a 5-pheromone blend: 90.05 Z11-14Ac:1.89 E11-14Ac:1.42 Z11-14OH:0.95 Z11-14Al:5.69 Z9-14Ac.

⁵ Ratio of components in a 2-pheromone blend: 94 Z11-14Ac:6 Z9-14Ac. SE: 3.68.

tions (*p*) of males responding to sources in the wind tunnel. ANOVA and the Tukey test were used to compare transformed proportions {arcsin[square-root(*p*)]} of the total captures per treatment per block in traps, in the field trial, P = 0.05 (Zar, 1996).

There were no significant differences in males staying or leaving the platform among treatments (Table 1). Source contact, however, was significantly different depending on sources ($\chi^2 = 16.92$, df = 6, P < 0.005). Responses to the attracticide containing 16% of the *P. pyrusana* 2-pheromone blend were significantly greater than any other source. The 16% 5-pheromone blend was not significantly different from the 0.16% of the *P. pyrusana* 2-pheromone blend, but was significantly greater than the 0.16% 5-pheromone blend.

Statistical differences were observed in *P. pyrusana* males total captures in the field in traps (Fig. 1) baited with a grease formulation containing the 2-pheromone blend or the 5-component blend at 1.6% or 16% concentrations (F = 19.33, df = 3,9, P < 0.001). These results agree with indoor bioassay data, i.e. captures in traps baited with 16% of the *P. pyrusana* 2-pheromone blend were significantly greater than other sources loaded with lower pheromone concentrations. Besides, in the field trial the 16% 5-pheromone blend source was not significantly different from the 1.6% of the *P. pyrusana* 2-pheromone blend, but was significantly greater than the 1.6% 5-pheromone blend.

The male approach to, and contact with the 16% two-pheromone blend source in a wind tunnel, was similar as they behave to females (Curkovic *et al.*, 2006). However, the responses were different and reduced to any other evaluated source. Since it has been already demonstrated that lower concentrations of the conspecific blend significantly reduce the response in *P. pyrusana* males to sources (Curkovic and Brunner, 2006), the results reported in here (the 2- vs. the

5-pheromone blend at the same concentration) suggest that the *C. rosaceana* pheromone components added into sources act as an inhibitor for *P. pyrusana* males. It remains unclear, however, whether one or more minor *C. rosaceana* pheromone component(s), or other unknown mechanism, elicit this response. This effect is undesirable for a simultaneous attracticide approach for both species. Interestingly, the inhibitory effect decreased using the same blend but increasing pheromone concentration at the source.

The behavioral analysis indicates that only the last step (source contact) within the *P. pyrusana* male searching sequence was significantly reduced. A possible explanation for this type of response would be due to the activity of minor components, found once the male has already took off, as indicated by Sweeney

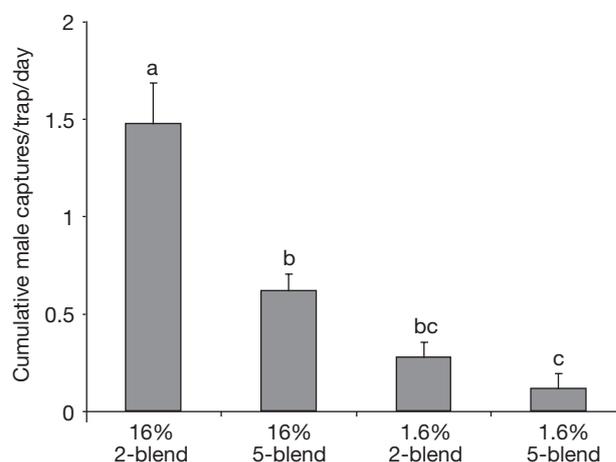


Figure 1. Total captures of male *Pandemis pyrusana*/trap/day in Delta traps (n = 4) baited with a grease containing 16% or 1.6% of a 2-pheromone blend (94 Z11-14Ac:6 Z9-14Ac) vs. sources containing a 5-pheromone blend (90.05 Z11-14Ac:1.89 E11-14Ac:1.42 Z11-14OH:0.95 Z11-14Al:5.69 Z9-14Ac), Wenatchee, Washington, USA, from August 26 to September 6, 2002, ANOVA and Tukey test (P = 0.05). SEM bars are included.

et al. (1990). The inhibitory effect observed probably helps to avoid interspecific matings in nature. Overall, results are the first report for *P. pyrusana* pheromone inhibitors which might be useful to control this species, but do not support the development of an attracticide targeting *P. pyrusana* and *C. rosaceana*.

References

- ARN H., 1991. Sex pheromones. In: Tortricid pests (Van deer Geest L.P.S., Evenhuis H.H., eds). Elsevier, Amsterdam, The Netherlands. pp. 187-207.
- CARDÉ R.T., HAGAMAN T.E., 1979. Behavioral response of the gypsy moth in a wind tunnel to air-borne enantiomers of disparlure. *Environ Entomol* 8, 475-484.
- CURKOVIC T., 2004. Behavioral responses of *Choristoneura rosaceana* (Harris) and *Pandemis pyrusana* Kearfott males to attracticide sources. Doctoral Thesis, Washington State University, Pullman, USA.
- CURKOVIC T., BRUNNER J.F., 2003. Evaluación de una formulación atrácticida para controlar *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) en manzanos en el estado de Washington, EE.UU. *Agric Tec (Chile)* 63, 231-239. [In Spanish].
- CURKOVIC T., BRUNNER J.F., 2005. Residual and sublethal effects of an attracticide formulation on *Choristoneura rosaceana* (Harris), *Pandemis pyrusana* Kearfott, and *Cydia pomonella* (L.) males (Lepidoptera: Tortricidae). *Crop Prot* 24, 637-641.
- CURKOVIC T., BRUNNER J.F., 2006. Evaluation of permethrin for attracticide development against *Choristoneura rosaceana* (Harris) and *Pandemis pyrusana* Kearfott males (Lepidoptera: Tortricidae). *Crop Prot* 25, 973-976.
- CURKOVIC T., BRUNNER J.F., LANDOLT P.J., 2006. Courtship behavior on *Choristoneura rosaceana* and *Pandemis pyrusana* (Lepidoptera: Tortricidae). *Ann Entomol Soc Am* 99, 617-624.
- EVENDEN M., McCLAUGHLIN J.R., 2005. Male Oriental fruit moth response to a combined pheromone-based attracticide formulation targeting both Oriental fruit moth and codling moth (Lepidoptera: Tortricidae). *J Econ Entom* 98, 317-325.
- FADAMIRO H.Y., COSSE A.A., BAKER T.C., 1999. Fine-scale resolution of closely spaced pheromone and antagonist filaments by flying male *Helicoverpa zea*. *J Comp Physiol A* 185, 131-141.
- GEMENO C., SANS A., LÓPEZ C., ALBAJES R., EIZAGUIRRE M., 2006. Pheromone antagonism in the European corn borer *Ostrinia nubilalis*. *J Chem Ecol* 32, 1071-1084.
- KNIGHT A.L., TURNER J.E., 1998. Sexual biology of *Pandemis pyrusana* (Lepidoptera: Tortricidae) under laboratory conditions. *J Entom Soc BC* 95, 89-94.
- KRUPKE C.H., ROITBERG B.D., JUDD G.J.R., 2002. Field and laboratory responses of male codling moth (Lepidoptera: Tortricidae) to a pheromone-based attract-and-kill strategy. *Environ Entomol* 31, 189-197.
- RENOU M., GUERRERO A., 2000. Insect parapheromones in olfaction research and semiochemical-based pest control strategies. *Annu Rev Entomol* 48, 605-630.
- ROELOFS W.L., LAGIER R.F., HOYT S.C., 1977. Sex pheromone of the moth, *Pandemis pyrusana*. *Environ Entomol* 6, 353-354.
- SWEENEY J., MCLEAN J., FRISKIE L., 1990. Roles of minor components in pheromone mediated behavior of western spruce budworm male moths. *J Chem Ecol* 16, 1517-1530.
- VAKENTI J.M., GAUNCE A.P., SLESSOR K.N., KING G.G.S., ALLAN S.A., MADSEN H.F., BORDEN J.H., 1988. Sex pheromone components of the obliquebanded leafroller, *Choristoneura rosaceana*, in the Okanagan valley of British Columbia. *J Chem Ecol* 14, 605-621.
- WITZGALL P., PRIESNER E., 1991. Wind-tunnel study on attraction inhibitor in male *Coleophora laricella* Hbn. (Lepidoptera: Coleophoridae). *J Chem Ecol* 17, 1355-1362.
- ZAR J., 1996. *Biostatistical analysis*. Prentice-Hall, Upper Saddle River, New Jersey, USA, 620 pp.