CHEMISTRY OF THE SEX PHEROMONE GLAND OF THE FALL WEBWORM, *HYPHANTRIA CUNEA*, DISCOVERED IN NEW ZEALAND

A.M. EL-SAYED, A.R. GIBB and D.M. SUCKLING

HortResearch, PO Box 51, Lincoln, New Zealand

Corresponding author: AEl-Sayed@hortresearch.co.nz

ABSTRACT

The sex pheromone glands of two females of the fall webworm, *Hyphantria cunea* Drury (Lepidoptera: Arctiidae), that were discovered in Auckland in March 2003 were extracted in hexane and were subjected to GC-MS analysis. Four compounds were identified, (9Z,12Z,15Z)-octadeca-9,12,15-trienal, (9Z,12Z)-octadeca-9,12-dienal, (3Z,6Z)-9S,10R-cis-epoxyhenicosa-3,6-diene, and (1,3Z,6Z)-9S,10R-cis-9,10-epoxyhenicosa-1,3,6-triene. The ratio of these four compounds in the gland extracts was 6.6:0.6:27.9:0.1. Except for a trace amount of the trienic epoxide detected in the New Zealand sample, the ratio of the three other compounds was very similar to native North American females of this species. A direct comparison between the synthetic commercial lure, Nitolure, and the sex pheromone gland of females found in New Zealand indicates that the sex pheromone gland contains significantly more of (3Z,6Z)-9S,10R-cis-epoxyhenicosa-3,6-diene than the synthetic lure, while the lure lacks the trace amount of the trienic epoxide. Therefore the effectiveness of Nitolure at trapping fall webworm in New Zealand remains uncertain.

Keywords: pheromone, Arctiidae, fall webworm delimitation, biosecurity.

INTRODUCTION

The fall webworm, *Hyphantria cunea* Drury (Lepidoptera, Arctiidae), is native to North America and Mexico. This insect feeds on hundreds of species of trees, shrubs, ornamentals and annual plants (Metcalf et al. 1962). The fall webworms weave their colonial webs at the ends of the branches to feed on foliage, while protected by their silken tent. Depending upon the climate, the fall webworm can have one to four generations per year. Outbreak populations of the fall webworm can completely defoliate host trees. Several consecutive defoliations can cause dieback in the crown, and may contribute to the death of weak, declining trees. This insect was accidentally introduced into Japan and southern Europe from North America in 1945 after World War II and now it is considered one of the most important pests of street and garden trees in urban areas in Japan. From there, it dispersed far to the East and invaded China, where it was first reported in 1979.

The chemistry of the sex pheromone of the fall webworm has been studied both in North America and Europe (Hill et al. 1982; Toth et al. 1989; El-Sayed 2005). Linoleic and linolenic aldehydes, (9Z,12Z)-octadeca-9,12-dienal (Z9,Z12-18Ald) and (9Z,12Z,15Z)-octadeca-9,12,15-trienal (Z9,Z12,Z15-18Ald), together with the dienic epoxide (3Z,6Z)-9S,10R-cis-epoxyhenicosa-3,6-diene (Z3,Z6-9S,10R-epo-21Hy) were found in the sex pheromone gland extracts of females originating from North America. The ratio of these three compounds in the sex pheromone gland of the North American females was 1:6:27 respectively (Hill et al. 1982). Although these three compounds were identified in the sex pheromone gland, no males were caught in the field with any combination of these compounds. Two further trienic epoxide compounds, (1,3Z,6Z)-9S,10R-cis-9,10-epoxyhenicosa-1,3,6-triene (1,Z3,Z6-9S,10R-epo-21Hy) and (1,3Z,6Z)-9S,10R-

cis-9,10-epoxyeicosa-1,3,6-triene (1,Z3,Z6-9S,10R-epo-20Hy), were later identified in the sex pheromone gland of female fall webworm originating from Hungary (Toth et al. 1989). Subsequently, a ternary mixture of Z9,Z12,Z15-18Ald, Z3,Z6-9S,10R-epo-21Hy and 1,Z3,Z6-9S,10R-epo-21Hy was found to be attractive to the male fall webworm in northern Italy (Trematerra et al. 1993). In China, Zhang et al. (1996) found that a ternary mixture of Z9,Z12,Z15-18Ald, Z3,Z6-9S,10R-epo-21Hy and 1,Z3,Z6-9S,10R-epo-21Hy at ratio of 8:1:1 captured the greatest number of males in field trapping experiments.

A single nest of fall webworm larvae (n=16) was discovered in Auckland, New Zealand, in March 2003. The potential threat of this insect to native and commercial plants was considered to be significant, due to its wide host range and invasive biology in other countries (Masaki & Umeya 1977). Therefore, an extensive pheromone trapping programme involving over seven hundred traps was proposed in order to monitor the situation in advance of any proposed control actions. From this collection of larvae, two adult females emerged in April 2003. As no others were available, it was deemed important to utilize these two individuals to analyze the chemical structure of their sex pheromone, and compare their pheromone with a commercially available synthetic lure, Nitolure (Nitto Denko, Osaka, Japan). It was expected that chemical identification of the sex pheromone would confirm the taxonomic identification and aid in the use of the sex pheromone for monitoring, detection and potentially eradication of this insect in New Zealand.

METHODS

Insects

Fall webworm larvae were collected from a sweet gum tree (*Liquidambar* sp.) at 132 B Aranui Rd, Mt. Wellington, Auckland in 2003 and were fed on leaves of *Liquidambar* sp. and maintained in a containment laboratory at 25°C and 12:12 h L:D until pupation and female emergence. Females were 2-3 days old at the time of sex pheromone extraction.

Sex pheromone gland and synthetic lure extraction

The sex pheromone glands of two females were combined together and were extracted in ca 20 µl of hexane at about 11 am. After 10 min, hexane was decanted from the glands, transferred into a new vial and maintained at -20°C until use. A synthetic lure was cut into small strips and extracted in 100 µl of hexane for 10 min and analyzed immediately by GC-MS.

Chemicals

The synthetic compounds Z9,Z12,Z15-18Ald, and Z9,Z12-18Ald were obtained for comparative purposes from the Institute of Pesticide Research at Wageningen, The Netherlands. The synthetic compound Z3,Z6-9S,10R-epo-21Hy was obtained from T. Ando (Tokyo University of Agriculture and Technology, Japan). The commercially available synthetic lure Nitolure produced by Nitto Denko, Osaka, Japan, and used in the trapping programme, was obtained and stored at -20°C until use. All synthetic compounds were > 97% pure by GC analysis and stored at -20°C until used.

Gas Chromatograph-Mass Spectrometry (GC-MS) analysis

GC-MS analysis of the gland extracts and synthetic lures were conducted on a Shimadzu QP5050A (Shimadzu, Kyoto) gas chromatograph mass spectrometer (GC-MS) using an ionization voltage of 70 eV and a mass range of 30 to 500. Separations were on a 30 m x 0.25 mm i.d. x 0.25 µm phase thickness, DB-5 capillary column (J&W, Folsom, CA, USA) or BPX-70 capillary column (SGE, Australia). The temperature of the MS ion source and the transfer line from the GC to the ion source was 240°C, and the GC injection port was at 220°C. Samples were injected in splitless mode with the split opened after 1 min. The oven temperature was maintained at 80°C for 2 min, raised to 240°C at a rate of 10°C/min, and held for 30 min. The head pressure (helium) was held at 50 kPa for 2 min, increased by 2 kPa/min to 90 kPa and held for 9 min. Compounds were identified by comparing their retention times and mass spectra of authentic standards on two different capillary columns to those of synthetic compounds or with spectra published in the literature.

RESULTS AND DISCUSSION

GC-MS analysis of the synthetic lure (Nitolure) indicated the presence of the following compounds: Z9,Z12,Z15-18Ald, Z3,Z6-9S,10R-epo-21Hy, and Z9,Z12-18Ald in a ratio of 12.3:1.1:1.2 (Table 1). The identity of the three compounds was confirmed by comparison with retention time and mass spectra of authentic standards on two different capillary columns. Nitolure has been commercially available in Japan since 1993. Traps baited with Nitolure were found to be extremely attractive to the male fall webworm in Japan and in North America (Shirou Satoda, pers. comm.). Based on the data provided by Nitto Denko (Shirou Satoda, pers. comm.), three active compounds are present in the synthetic lure: Z9,Z12,Z15-18Ald (>4.3 mg/lure), Z3,Z6-9S,10R-epo-21Hy (>0.58 mg/lure) and 1,Z3,Z6-9S,10R-epo-21Hy (>0.49 mg/lure). To reduce the cost, in their lures Nitto Denko uses impure Z9,Z12,Z15-18Ald, which contains Z9,Z12-18Ald (<2%). In our analysis, 1,Z3,Z6-9S,10R-epo-21Hy was not detected in the lure. However, this does not imply that this compound was not present. Its absence could be due to its thermal instability especially during the GC-MS analysis (S. Satoda, pers. comm.).

TABLE 1: Chemical composition and ratio of the sex pheromone gland of the female fall webworm, *H. cunea*, found in New Zealand, North America and in a synthetic attractant (Nitolure).

Compound	Female		Nitolure
	North America ¹	New Zealand	
Z3,Z6-9S,10R-epo-21Hy	27	27.9	1.1
Z9,Z12,Z15-18Ald	6	6.6	12.3
Z9,Z12-18Ald	1	0.6	1.2
1,Z3,Z6-9S,10R-epo-21Hy	not detected	0.1	not detected

¹Hill et al. (1982).

Four pheromone-like compounds were identified in the pheromone gland of the two female fall webworms found in New Zealand. These compounds were Z9,Z12,Z15-18Ald, Z9,Z12-18Ald, Z3,Z6-9S,10R-epo-21Hy and 1,Z3,Z6-9S,10R-epo-21Hy. Except for 1,Z3,Z6-9S,10R-epo-21Hy, the identity of the three compounds was confirmed by comparison with the retention times and mass spectra of authentic standards on two different capillary columns. The identity of 1,Z3,Z6-9S,10R-epo-21Hy was confirmed by comparison with a published spectrum (Toth et al. 1989). The ratio of these four compounds in the gland extracts was 6.6:0.6:27.9:0.1 (Table 1). Except for the trace amount of the trienic epoxide detected in our analysis, the ratio of the three other compounds was very similar to that found in the North American females that was reported by Hill et al. (1982) to be 6:1:27 (Table 1).

In this study, only two females were available for GC-MS analysis due to the small population found. There is no information in the literature on the variation in the pheromone chemistry among female fall webworms. Usually, in chemical identification studies ten females are used to give a good estimate of the composition of the sex pheromone gland (e.g. El-Sayed et al. 2005). Therefore, it is difficult to postulate how much these results represent the whole population. However, there is a striking similarity between these data and the data published by Hill et al. (1982), which might indicate that the variation between female fall webworms is limited. Also, this gives confidence that the small sample size did not affect the ability to accurately interpret the mass spectra data.

A direct comparison between the chemistry of Nitolure and the sex pheromone gland of females found in New Zealand indicates that they differ in the ratio of Z3,Z6-9S,10R-epo-21Hy, with the sex pheromone gland containing substantially more of this compound compared to Nitolure. In addition the New Zealand sex pheromone glands contained trace amounts of the trienic epoxide that were not found in Nitolure

(Table 1). It is difficult to postulate how the disparity in the ratio of the dienic epoxide between the sex pheromone glands of females found in New Zealand and *Nitolure* will influence the efficacy of the synthetic lure for monitoring the presence of the fall webworm in New Zealand. The significant trapping effort conducted over the last two years was predicated on the assumption of high trap efficiency at detecting the insect. Limited trapping trials were conducted in the field in the Eastern USA with *Nitolure* obtained by MAF from Japan in 2004, but only a very few adult male fall webworm were caught (a maximum of three per trap in two months; B. Fusco & S. Liebhold, pers. comm.). These results were inconclusive with regard to lure efficacy, due to possible issues with a lack of synchronicity between the trapping period and male peak flight, as well as potential cross contamination since one operator in this trapping experiment was in contact with a gypsy moth lure. Therefore, additional experiments are required to test various synthetic blends with various ratios of the four compounds identified in the female gland. Further field tests overseas would help to assess the efficacy of the *Nitolure* that is also being used in the New Zealand monitoring programme. The catch of four male fall webworms in Mt. Wellington in February and March 2005 (I. Gear, pers. comm.) indicates that the programme will have an ongoing need for accurate surveillance for some time. Another possible means to validate and possibly improve the efficacy and sensitivity of this lure for New Zealand surveillance purposes would be to establish a colony from any fall webworm larvae collected in New Zealand and conduct wind tunnel trials using *Nitolure*. However, no larvae have been found since 2003.

The linolenic aldehyde Z9,Z12,Z15-18Ald has been identified in the sex pheromone gland of many Arctiid species, including the Red hairy caterpillar, *Amsacta albisirga* (Walker), the Bihar hairy moth, *Diacrisia obliqua* (Walker) and the Saltmarsh caterpillar, *Estigmene acrea* (Drury) (El-Sayed 2005). The trienic epoxide, 1,Z3,Z6-9S,10R-epo-21Hy, has been identified in the sex pheromone gland of *D. obliqua* (Yadav et al. 2001). The dienic epoxide Z3,Z6-9S,10R-epo-21Hy is more common and present in the sex pheromone gland of many species belonging to the family Arctiidae, Lymantriidae, Geometridae and Noctuidae (El-Sayed 2005). Therefore, it is likely that a synthetic lure for *H. cunea* will be attractive to other moth species present in New Zealand. In experiments conducted in Lithuania, the synthetic pheromone of fall webworm was attractive to other species, e.g. the white ermine, *Spilosoma lubricipeda* (L.) (Arctiidae), the common pug, *Eupithecia vulgata* (Haworth) (Geometridae) and the large yellow underwing, *Noctua pronuba* (Hufnagel) (Noctuidae) (Ostrauskas 2004). In Italy, the synthetic pheromone of fall webworm was attractive to another arctiid species, the Buffer ermine, *Spilosoma luteum* (Hufnagel) and the Noctuidae *N. pronuba* (Maini & Belifiori 1996). Unfortunately, none of these lures is available for further study due to a lack of commercial supply.

In spite of the striking similarity in the ratio of three of the four compounds found in the sex pheromone gland of two females discovered in New Zealand compared to the North American population (Hill et al. 1982), this does not imply that the females discovered in New Zealand originated from North America. This is because there is no published information on the composition of the sex pheromone gland of the fall webworm population in Japan, and it is thought the Japanese population originated from North America. However, the very close similarity in composition of the sex pheromone gland between females discovered in New Zealand and the published pheromone of the fall webworm confirms that the females discovered in New Zealand are the fall webworm, *H. cunea*.

Sex pheromone baited traps are an essential tool for monitoring and detection of invasive species. Therefore, optimization of the pheromone blend for this invasive species will enhance the ability to monitor and hence eradicate this pest in New Zealand. In addition to monitoring and detection, sex pheromones are being used to control insect pests in many different ways, including mating disruption, mass trapping and lure and kill (Suckling 2000), and have an increasing role in biosecurity (Suckling & Gibb 2003). Mating disruption has been used successfully for suppression of many agriculture pests. However, this approach might not be feasible against the fall webworm, because it

requires a large amount of pheromone to be applied and the pheromones of this insect are very expensive. In addition, it is likely that this approach will not work in urban areas due to the presence of obstacles and buildings. Mass trapping trials have been conducted in Japan against the fall webworm (Yamanaka et al. 2001; Satoda 2002). Nitolure was used in these trials, but there are contradictory reports regarding the efficacy of this technique against the fall webworm. In some trials mass trapping does not reduce the damage (Yamanaka et al. 2001), while in other trials the approach provided an effective control method against the fall webworm (Satoda 2002). Another possible application of the sex pheromone for the eradication of fall webworm is through the use of lure and kill. However, this approach will first require the efficacy of the available lure to be demonstrated and there are other operational factors to be dealt with in order to achieve effective results.

ACKNOWLEDGMENTS

This study was funded by the Foundation for Research, Science & Technology (FRST), and New Zealand Ministry of Agriculture and Forestry. We are grateful to Alan Flynn for supply of the insects, and to Sandy Liebhold (USDA) and Bob Fuscoe (Abbott Laboratories) for trapping information. We also thank Dr T. Ando (Tokyo University of Agriculture and Technology, Japan) for supplying a chemical sample.

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