

ALTERNATIVE DISPENSING TECHNOLOGIES FOR PHEROMONE MATING DISRUPTION OF CODLING MOTH IN POME FRUIT AND NUT CROPS

Stephen C. Welter^{1*} and John E. Dunley²

¹University of California, Berkeley, CA, USA,

²Washington State University, Wenatchee, WA, USA

Two dispensing technologies present promising alternatives to conventional hand-applied pheromone dispensers currently in wide usage in California, USA. Pheromone mating disruption of codling moth is currently being used on approximately 50% of the pome fruit orchards in the western United States north of Sacramento. While these programs have produced economically acceptable control of less than 1% damage on average, the relatively high cost and logistic difficulties associated with hand application has encouraged the development of alternative dispensing technologies. Two of these technologies include aerosol dispensers that emit high levels of pheromone from few point sources in the orchard as well microencapsulated sprayable formulations. The relative area of influence for the aerosol emitters was estimated indirectly using pheromone trap suppression as an indirect indicator. Uniform distributions of codling moth within an orchard were achieved by releases of sterile, marked codling moth within a uniform grid. Similarly, grids of recapture grids were established over the orchards. Indirect imaging of the areas of influence suggested that a single point source was capable of suppressing traps up to 600 meters downwind of the emitter, whereas lateral spread of 100-150 meters was also observed with downwind movement. Virtually no lateral spread was observed from emitters placed along the upwind edges of orchards. Using similar approaches, residual effects downwind from the emitters lasted for several days after the unit was no longer functioning. Fruit damage was evaluated in 2001 using a similar approach of mapping relative to the trap and release grids.

Impressive results for trap suppression were also observed for several formulations of sprayable microencapsulated pheromones for codling moth. Single applications were capable of suppressing traps for more than 60 days using products by 3M or Consep. Rate responses between trap suppression and application rate were extremely flat using replicated plots of approximately 1 ha per plot. The potential cost savings and ease of application may provide strong incentives to switch to these alternatives if damage suppression proves efficacious.

ATTRACT AND KILL TECHNOLOGY: A “KNOCK-OUT” IN APPLICATION OF PHEROMONES FOR PEST MANAGEMENT

Philipp Kirsch

IPM Technologies USA

Monitoring traps, mating disruption, and attract and kill baits are the principal methods that have been developed to exploit insect pheromone behaviours for pest management objectives. As with any management tool, the operational use of pheromones must be considered within the context of an integrated pest management system. The successful operational deployment of mating disruption tactics almost always requires judicious application of supplementary insecticides, as predicted by Pickett (1991) in that “pheromones alone will..... be insufficiently robust for the purposes of most agricultural production systems”.

A more recent approach, Attract and Kill (A&K), has been developed. This technique uses a semiochemical, such as the sex pheromone or a feeding attractant, in combination with an insecticide. Thus, when adults contact the lure they succumb to the toxic effect of the insecticide. A&K systems are more powerful than other semiochemical mediated control strategies such as mating disruption in that male moths are incapacitated and removed from the ecosystem. Dead males cannot mate. Yet, this approach has the obvious advantage of limiting any potential negative ecological effects of the insecticide, as only those insects coming to the lure will be affected.

Commercialization of the A&K approach has been undertaken by IPM Technologies Inc. who have global rights to a proprietary and patented matrix combining insecticide and attractant in a UV sensitive carrier material. Marketed as Sirene and Last-Call in Europe and the U.S., respectively, this technology was granted US EPA registration in 1998 and California registration in 1999. The robust matrix can accept, protect and release a wide variety of chemicals (acetates, alcohols, aldehydes) so it has the potential to be deployed against many pest species in diverse ecosystems.

Pheromone-based Last Call products have been commercialized against codling moth in apples and pears, potato tuber moth in solanaceous vegetables, false codling moth in citrus, litchii and other subtropical fruits, and pink bollworm and boll weevil in cotton. Experimental trials show promise against pine shoot borers (*Eucosma* and *Rhyacionia* in pine plantations), pecan nut casebearer in pecans, leafrollers in deciduous fruit and vines, loopers and fruitworms in vegetables, diamondback moth in crucifers and douglas fir cone gall midge in fir seed orchards.

PRACTICAL USE OF PHEROMONE MATING DISRUPTION

Kinya Ogawa¹, Toshimi Kobayashi^{1*} and Takehiko Fukumoto²

¹Shin-Etsu Chemical Co. Ltd. Fine Chemical Department

²Shin-Etsu Chemical Co. Ltd.
Specialty Chemicals Research Center

We have developed and sold pheromone dispensers for mating disruption over 17 years in many countries. This method has been highly successful in the field. However, in some cases, our collaborators have not been successful. Here, we will explain some successful cases and analyze the reasons for success. The analyses of these cases show us the important points in using this method. Mating disruption is useful and cost effective when farmers use pheromones as the main means of IPM.

The important points are as follows:

1. Choose pests that live in or covered by plant tissue during the larval stage
For example, *Synanthedon hector* Butler
2. Choose a good dispenser design
Uniform release; long life
3. Apply a suitable quantity of active ingredient
Sufficient concentration of pheromone in the air
4. Apply at an appropriate time
Late application is a major reason for failure
5. Protection of natural enemies
The main idea of using mating disruption in IPM
6. Treat large areas
To avoid edge effects

The cost of pheromone dispensers is rather expensive when compared in isolation with the cost of pesticides used against a target pest. However, when using mating disruption in an IPM program, natural enemies are able to work effectively. This means that farmers can reduce the use of acaricides and insecticides which would be required for secondary pests which occur in the absence of natural enemies. As a result, the cost of the total plant protection program is cheaper when compared with the conventional system. This point is important in practical farming.

**INTRA- AND INTER-SPECIFIC ATTRACTION, MATING BEHAVIOUR AND
HYBRIDIZATION BETWEEN *BACTROCERA CARAMBOLAE* AND *B. PAPAYAE*.**

Suk-Ling Wee

School of Biological Sciences
Universiti Sains Malaysia
Minden 11800
Penang, Malaysia

Fruit flies, *Bactrocera carambolae* and *B. papayae* are two economically important species infesting many commercially grown fruits in Malaysia, Indonesia and Thailand. The responses of *B. carambolae* and *B. papayae* females to conspecific and heterospecific males were studied in a wind tunnel during courtship period. During peak courtship period, *B. carambolae* female attraction to heterospecific male was not significantly different from conspecific male. However, *B. papayae* female demonstrated a higher attraction to conspecific than heterospecific males. These sibling species exhibited a similar sequence of courtship behaviour and possessed an overlapping courtship period. However, *B. papayae* male preceeded *B. carambolae* in sexual excitation at a higher light intensity and spent longer time in courting the females. When engaged in courtship, *B. papayae* male were observed to be more aggressive than *B. carambolae* males. Likewise, *B. papayae* female was also observed to be more responsive to male calling than *B. carambolae* female. Most of the intra- and inter-specific copulation lasted for more than 9 hours. Biological parameters, such as egg production, immature development and adult sex ratio of the intra- and inter-specific mating of *B. carambolae* and *B. papayae* were obtained and compared. While *B. papayae* matured *ca.* 2 weeks earlier than *B. carambolae*, sexual maturation rate of hybrids of the reciprocal crosses was between that of the parents. Morphology of the hybrids ranged from typical parental characteristics to intermediate characteristics between the two species. Similar results were also obtained for hybrid male and female genitalia length when compared with the parental species. Pheromonal analysis revealed that hybrid possesses a more complicated combination of pheromonal components compared with those of their parents.

DETECTION OF AROMATIC COMPOUNDS FROM THE CELL SUSPENSION CULTURES AND THE *IN VITRO* PLANTLETS OF *CYMBOPOGON NARDUS*

Chan Lai Keng^{1*}, Patra R. Dewi¹, S.K. Lai¹ and P.L.Boey²

¹ School of Biological Sciences

² School of Chemical Sciences

Universiti Sains Malaysia

11800 Penang, Malaysia

Cymbopogon nardus (serai wangi) could be mass propagated via *in vitro* culture method by culturing the axillary buds in proliferating medium consisting of Murashige and Skoog medium salts (MS) supplemented with 0.3 mg/L benzyladenine (BA) and 0.1 mg/L indole-3-butyric acid (IBA). An average of 4.7 shoots were produced from each axillary bud within four weeks. These *in vitro* plantlets were found to contain 33% citronellal, 47.7% geraniol and 19.1% citronellol as compared to the mother plants which contained 49% citronellal, 30% geraniol and 14.2% citronellol. The cell suspension cultures of *Cymbopogon nardus* were prepared by culturing 0.5 g callus tissues into 20 ml MS liquid medium containing 2 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D) and an increased wet weight of 0.23 ± 0.11 g of cells were obtained after 15 days of culture. Geraniol and citronellol were the main aromatic compounds in the cell suspension cultures of *Cymbopogon nardus*.

IMPROVED SEMIOCHEMICALS FOR FRUIT FLY DETECTION AND CONTROL

Eric Jang

U.S. Pacific Basin Agricultural Research Center

Semiochemicals are the basis for most tephritid fruit fly detection and delimitation programs, and in some cases used in control and eradication of introduced exotic pest species. Most notable are the male attractants methyl eugenol and cue lure which are powerful lures for several *Bactrocera* species throughout South East Asia and the Pacific. Trimedlure, the attractant for Mediterranean fruit fly is less attractive on a relative scale than either of the lures for the *Bactrocera* species. Although the precise role that these semiochemicals play in the ecology of the species has not been fully elucidated, several new interesting discoveries have furthered our understanding of the possible role these chemicals play in intraspecific behaviors.

In collaboration with chemists at the USDA-ARS, Insects Affecting Insect Behavior Laboratory in Beltsville, MD, we have been evaluating two improved semiochemicals which appear to be more attractive than their predecessors. Minus B1 Ceralure is a stereospecific enantiomer of Ceralure, an iodo derivative of Trimedlure. This compound appears to be significantly more attractive than Trimedlure in field tests using released laboratory-reared as well as wild-type flies. An additional benefit is that attraction last longer than with Trimedlure and significantly

less material is needed to obtain an equivalent attractiveness. We have also tested a formate derivative of cue-lure which appears to be more active than cue-lure. The formate captures more male melon flies than cue-lure and is thus a good candidate for detection and possible control of melon fly. While synthesis of the formate appears to be achievable, the synthesis of the minus B1 Ceralure is difficult at this time. These semiochemicals may further improve detection of these species and could lead the way to their use in control programs.

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**ASSESSMENT OF USING SEX PHEROMONE FOR CONTROL OF THE
CARAMBOLA FRUIT BORER, *EUCOSMA NOTANTHES* MEYRICK, AT
CARAMBOLA ORCHARDS**

Hung, Chau Chin^{1*}, Roger F. Hou² and Jenn Sheng Hwang¹

1. Taiwan Agricultural Chemicals and Toxic Substances Research Institute (TACTRI)
Council of Agriculture, Wufeng
Taichung 413, Taiwan, R.O.C.
2. Department of Entomology, National Chung Hsing University
Taichung, Taiwan, R.O.C.

The carambola fruit borer (CFB), *Eucosma notanthes* Meyrick, is the key pest attacking carambola fruits in Taiwan. Assessment of using sex pheromone for monitoring, mass trapping, and mating disruption of CFB was studied in the orchards. Monitoring tests were conducted at Yuan-lin and Cho-lan areas from 1990 to 1992. CFB occurrence in these two areas was similar, showing lower population density from April to June. The population density in Cho-lan was significantly lower than that in Yuan-lin. Mass trapping was conducted at Guo-xing, Nan-tou county from 1993 to 1997. CFB density declined to 1.0-4.5 insects/trap/week when 40-80 traps/ha were used. CFB population density was reduced by 75.4-89.4% compared to untreated orchards, and the fruit damage was only 1.9%. There was no difference in the effectiveness of mating disruptants formulated with Z8-12:Ac from different sources. In order to control CFB by mating disruption, a 5- hectare orchard at Cho-lan was treated with 53.28 g/ha of Z8-12:Ac disruptants for 5 months during 1996-97. Percent inhibition of male attraction reached 100%. The mating inhibition in the cage experiment and carambola orchards was 89.4 and 97.45%, respectively. The fruit damage was reduced by 71% as compared to conventional chemical control.

**TRAPPING AND MATING DISRUPTION OF THE DIAMONDBACK MOTH,
PLUTELLA XYLOSTELLA (L.) USING SEX PHEROMONES**

Syed A.R.^{1*}, Unal I.², Loke W. H.³ and Sivapragasam A.⁴

¹MARDI, Cameron Highlands, Pahang

²Universiti Kebangsaan Malaysia, Bangi, Selangor

³CABI, Southeast Asia Regional Office, Serdang, Selangor

⁴Strategic Research Center, MARDI, Serdang, Selangor

Studies were done to evaluate the trapping efficiency and the feasibility of mating disruption using sex pheromones of the diamondback moth (DBM), *Plutella xylostella* (L.). In the first study, the trapping efficiency of six commercially-available pheromone trap designs, viz, the Halex Trap I (wing type, white color); the Halex Trap II (wing type, yellow color); the BCS Trap I (cross-line wing type, white color); the BCS Trap II (delta type, white color); the IPS Trap (delta type, yellow color) and the Hoechst Trap (delta type, green color) were evaluated in cabbage fields during the dry and wet season at three different localities in Cameron Highlands. Each trap, hung about 60 cm above the ground, had a rubber septum-based lure impregnated with the Halex DBM sex pheromone blend. Weekly records of trapped DBM and parasitoids were made and the lure and the sticky bottom component of the trap were replaced as necessary. In the second study, four commercial pheromone lures were evaluated for their effectiveness to attract DBM in cabbage fields during the dry and wet season in Kea Farm, Cameron Highlands. The procedures to set up the traps and data collection were as described previously. In the third study, the mating disruption of DBM using sex pheromones was conducted in two commercial cabbage fields of 0.3 ha each. One of the fields was treated with the Konaga-Con type rope dispenser which contained the DBM sex pheromone at a concentration of 25g a.i. per 100m. Three hundred and fifty meters of the rope dispenser was suspended 50cm above the ground, over the cabbage canopy at 10m intervals to cover the whole area. The male DBM adults trapped were recorded weekly from five pheromone traps placed in the treated and untreated fields. In addition, weekly counts of DBM larvae and pupae on 120 cabbage plants were made.

In the first study, the number of DBM caught in all traps at all the locations was higher in the dry season than those in the wet season. This suggested that rainfall could be one of the major mortality factor for DBM. In the drier months, all the traps were efficient in capturing male DBM adults. In the wet months, when populations were lower, the Halex I, Halex II, IPS and BCS I trapped higher numbers of male DBM. It was interesting to note that all the traps also attracted *Diadegma semiclausum*, and that the two yellow traps i.e. Halex II and IPS significantly trapped higher numbers of *D. semiclausum* than the white traps. In the second study, the mean number of DBM captured per trap in the dry season was also higher than in the wet season and catches in the former decreased as follows: Hoechst (361.88) > BCS (351.75) > Halex (169.87) and IPS (16.12). In the wet season, the values were: Hoechst (55.38) > BCS (36.50) > Halex (21.75) and IPS (7.50). This showed that the Hoechst and BCS pheromones were relatively efficient in attracting male DBM. In mating disruption studies, the number of DBM adults was greatly reduced in the treated cabbage field as compared to the untreated 'check' field. At the termination of the study i.e. 45 days after mating disruption treatment, only 4.54 DBM adult males were recorded in the pheromone trap in the treated field compared to 92.61 DBM adults in the untreated field. There was also a 76 per cent reduction in the combined

larvae and pupae populations in the treated field. The study suggested that the mating disruption technique could be used as a tool in the integrated pest management program of DBM.

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**SEX PHEROMONE COMPONENTS OF THE JAPANESE PERSIMMON MOTH,
STATHMOPODA MASINISSA
(LEPIDOPTERA: STATHMOPODIDAE).**

H. Naka^{1*}, K. Tsuchida¹, H. Sakurai¹, H. Yamazawa², R. Yoshida²
T. Ando², T. Kimura³, H. Honda³

¹ Laboratory of Entomology, Faculty of Agriculture, Gifu University

² Graduate School of Bio-Applications and Systems Engineering, Tokyo University of
Agriculture and Technology

³ Laboratory of Applied Entomology and Zoology, Institute of Agriculture and Forestry,
University of Tsukuba

The Japanese persimmon moth, *Stathmopoda masinissa*, is one of the pests which damages Japanese persimmon fruits, and its sex pheromone has not yet been identified. We will report here a part of the identification of sex pheromone of this species.

One to four-day-old virgin females show a calling posture about 8-11h after the beginning of the scotophase. Males show a mating behavior during the corresponding time. We extracted sex pheromone gland extract about 9 after the beginning of the scotophase from 1 to 4-day-old virgin females showing a calling posture upon the extraction.

The pheromone gland extract prepared from 1,500 virgin females showed three distinct EAG-active components (A, B and C) in the GC-EAD analysis. GC-MS data indicated that component A is a C16 aldehyde with two conjugated double bonds and components B and C are its acetate and alcohol derivatives.

The GC-MS data of component A indicated an unusual base peak at m/z 84, so we estimated the double bond position of the component A and synthesized the corresponding compounds with the 4,6-diene function.

The GC-MS data of the synthetic standards with an E4, Z6 configuration matched well with those of the three natural pheromone components. We will synthesize other isomers and evaluate the attractiveness of each synthetics to the wild male moths.

**SEX PHEROMONE COMPOSITION OF THE SUMMER FRUIT TORTRIX,
ADOXOPHYES ORANA IN KOREA**

Kyeung Sik Han*, Jong Ho Park and Kyung Saeng Boo

Graduate School of Agricultural Biotechnology, Seoul Natl. Univ., Korea

The summer fruit tortrix, *Adoxophyes orana* has been claimed as the major leafroller on apple trees in Korea. Most *A. orana* mated before lights-on under the photoperiod of 16L:8D and the concentration of sex pheromone components peaked at the mating period although the major components were detected throughout the day. GC-MS and GC-EAD analysis of its sex pheromone gland extracts revealed the two main known compounds, (Z)-9-tetradecenyl acetate (Z9-14:Ac) and (Z)-11-tetradecenyl acetate (Z11-14:Ac). In EAG assay, Z11-14:Ac and Z9-14:Ac elicited significant antennal responses in male moths, but no other compounds, which have been previously reported for species of *Adoxophyes*, did. GC analysis showed that *A. orana* sex pheromone is composed of Z11-14:Ac and Z9-14:Ac with the ratio of 95:5. In field trapping tests conducted at apple and peach orchards in Korea, *A. orana* males were attracted mainly to 95:5 blend, before the dawn.

POSTERS

***IN VITRO* PROPAGATION OF *SPILANTHES ACMELLA*, A BIO-INSECTICIDE HERB**

Chan Lai Keng* and Ang Boon Haw

School of Biological Sciences
Universiti Sains Malaysia
11800 Penang

Spilanthes acmella was found to contain chemical compounds with insecticidal properties. These plants were successfully micropropagated using axillary buds as explants. Multiple shoots were formed within five weeks when the aseptic axillary buds were cultured in Murashige and Skoog medium (MS) supplemented with 2.0 mg/L N⁶-benzyl adenine (BA). The addition of indole-3-butyric acid (2.0 mg/L) had no significant effect on the multiple shoots formation of these plants. This study indicated that a MS medium supplemented with 0.5 mg/L BA was sufficient for the production of rooted multiple shoots of *Spilanthes acmella*. Repeated subculturing of the *in vitro* individual shoot in the same medium increased the formation of multiple shoots by three fold.

DETECTION OF JUVENILE HORMONE III FROM *IN VITRO* *CYPERUS AROMATICUS*

Chan Chee Weng* and Chan Lai Keng

School of Biological Sciences
Universiti Sains Malaysia

An *in vitro* culture system was developed for *Cyperus aromaticus* using buds from the rhizomes as explants. The main problem encountered in the *in vitro* culture of *Cyperus aromaticus* was contamination of cultures mainly by fungus and bacteria. Aseptic explants could be obtained by surface sterilization with solution contain 1.0 g/l mercuric chloride plus 300 mg/l sodium dichloroisocyanurate. To maintain an aseptic culture, the buds were cultured in solid Murashige and Skoog (MS) medium supplemented with 1.0 g/l potassium permanganate and 100 mg/l kanamycin for one week before transferring to the proliferate medium. MS medium supplemented with 0.5 mg/l indole-3-butyric acid (IBA) and 1.0 mg/l 6-benzyladenine (BA) was found to be the best medium for the production of multiple shoots. Rooting of the *in vitro* shoots was induced in basic MS medium. Detection of Juvenile hormone III from the *in vitro* plantlets was compared with that obtained from its natural habitat.

IMPROVED NEW MALE ATTRACTANT FOR MELON FLY

Eric Jang

USDA-ARS-PBARC

There is always a need for improvements of attractants used in monitoring and control programs of fruit flies. The current male attractant for melon fly is Cue-lure, an acetate derivative of raspberry ketone. Because of the limitations of Cue-lure as a result of its low volatility, some research has been conducted on other analogs of raspberry ketone. This research reports the results of a compound, which was shown to be 1.5 to 2 times more attractive to male melon flies than Cue-lure. The development of an improved melon fly attractant will provide a better tool for control of melon fly populations.

USE OF PHEROMONE MATING DISRUPTION AS A TACTIC FOR INSECTICIDE RESISTANCE MANAGEMENT IN PEAR AND APPLE

John E. Dunley^{1*}, Elizabeth H. Beers¹, Stephen C. Welter² and Jay F. Brunner¹

¹Tree Fruit Research and Extension Center, Washington State University

²University of California, Berkeley

The evolution of insecticide resistance in pests of deciduous tree fruits, apple and pear specifically, has long confounded the development of stable integrated pest management programs. Resistance has occurred in codling moth, the key pest of apple and pear, to most classes of insecticides used for its control. Additionally, insecticide resistance has occurred in tortricid leafrollers, also important economic pests of pome fruits. Resistance has occurred in these pests not only to the older insecticide classes, such as organophosphates and pyrethroids, but also to new insecticides with novel modes of action. These newer insecticides include growth regulators with varied activities such as chitinase inhibition, juvenile hormone analogs, and ecdysone agonism.

The development of mating disruption for codling moth and leafrollers has provided a new tactic for resistance management in the western US. A project implementing large-scale mating disruption for codling moth was initiated in 1993 in California pear. This project examined both the feasibility of mating disruption as an IPM tactic, and resistance management for organophosphate resistant populations of codling moth. The Codling Moth Areawide Management Project, funded by USDA-ARS, implemented mating disruption in Washington, Oregon, and California in 1995. These pilot projects were successful in leading to widespread implementation of mating disruption, such that by 2000 more than half of the apple and pear acreage in Washington were using this tactic.

Widespread implementation of codling moth mating disruption allows for unique resistance management. Along with overall reduction in codling moth densities, organophosphate resistance has decreased significantly. Reduced pest pressure has permitted use of chemical control tactics such as IGR's that are more environmentally-benign but relatively less effective than organophosphates. Reduction in broad-spectrum insecticide use has also provided more opportunities for biological control.

Mating disruption for leafrollers is currently under development. One large-scale implementation project, initiated in 1997, has been successful in apple. As with codling moth, leafroller mating disruption provides unique opportunities for developing long-term, stable IPM systems. Synthesis of mating disruption for multiple pests, and integration into resistance management and IPM systems, provides challenges and opportunities in the future.

**CONTROLLING TREE FRUIT PESTS WITH LAST CALL:
INNOVATIVE ATTRACT AND KILL TECHNOLOGY.**

Philipp Kirsch*, Gerhard Booyesen and Dariusz Czokajlo

IPM Technologies USA and Insect Science South Africa

Last Call is a novel pheromone/insecticide bait matrix that exploits the attract & kill concept to interrupt the life cycle at the adult moth stage. The product is applied in drops which imitate a female moth based on an equivalent release of sex pheromone in purity and dose. Attracted to this female decoy, the male tries to mate with Last Call and inevitably comes into contact with a lethal dose of insecticide. Following 10 years of development against codling moth (CM) in Switzerland and South Africa, and US EPA registration in 1998, product evaluation trials were established in commercial apple and pear orchards in 1999 in California, Oregon, Washington and British Columbia. This ready to use, highly viscous slow release formulation of Last Call CM, containing 0.16% pheromone (EE-8,10-12OH) and 6% permethrin, is applied to wood in the tree canopy at 1200 droplets/acre (3000/ha), with a uniform spacing throughout the treated orchard. Fields are retreated at 5 weeks.

In the 1999 trials, the cumulative total codling moth trap catches were considerably reduced in Last Call CM treated plots compared with conventional plots (707 males in 132 traps in Last Call vs 4466 males in 87 traps in conventional); and almost equally suppressed in comparison to disruption plots. Fruit damage was reduced in Last Call CM treated plots compared with both conventional and mating disruption plots (0.35% average in Last Call vs 1.75% in conventional plots).

Other trials show considerable promise for multi-species formulations targeting codling moth and leafrollers in a combination product, and for other tree fruit species including false codling moth, California red scale and Mediterranean fruit fly on citrus.

Last Call CM successfully controlled codling moth under diverse climates and geographic conditions (California to British Columbia), in orchards with high population pressures, uneven slopes and canopies, small size and irregular shape. The waterproof grease is effective for 5-7 weeks under overhead irrigation systems. Isolation is of minor importance as long as neighboring orchards are conventionally treated. The Last Call matrix has considerable utility for development of selective, effective and residue-free tools targeting tree fruit.

**LAST CALL ATTRACT AND KILL TECHNOLOGY
SHOWS PROMISE IN VEGETABLE PEST MANAGEMENT.**

Gerhard Booysen¹, Everett Mitchell², John McLaughlin¹, Dariusz Czokajlo¹
and Philipp Kirsch^{1*}

¹IPM Technologies USA

²USDA-ARS-IABBRL, Gainesville, Florida

Last Call, a novel pheromone/kairomone + insecticide gel, offers considerable utility in management of insect pests, using the attract & kill concept to interrupt the life cycle at the adult moth stage. The product is applied in drops imitating either a female moth (sex pheromone as bait) based on an equivalent release of sex pheromone in purity and dose, or the host plant (host volatiles as bait). Attracted to the bait, the adult insect inevitably comes into contact with a lethal dose of insecticide. Pheromone- and kairomone-based formulations were tested against vegetable pests in Florida, South Africa, Canada and Australia.

Commercial efficacy was proven in management of potato tuber moth on tomato in Southern Africa with a ready to use, highly viscous slow release formulation containing 0.16% pheromone (PTM I & PTM II) and 6% permethrin, dispensed at 100g/ha or 200g/ha onto the lower half of the plant as precise 50 ul droplets, for a total of 2000 or 4000 evenly spaced droplets/ha. Retreatment interval was 28 days. Moth flight was measured with traps and damage was measured by sampling leaves and fruit within Last Call treated and insecticide comparison plots. Pheromone trap capture was most suppressed, and fruit protection was highest in plots treated with the higher dose rate of 4000 droplets/ha or 200g/ha. Beneficials were not affected by the Last Call treatments. Compared with the conventional insecticide program, Last Call PTM was a superior control tactic.

Other species: Soybean and Cabbage Looper male and female moths were attracted to floral volatiles formulated into Last Call. Beet armyworm trap captures were reduced up to 100% over 19 days in small plots treated at 1450 droplets/acre. Diamondback Moth: dead moths were easily found on leaves in cabbage plots treated with Last Call formulated with pheromone. Male and female *H. armigera* moths were attracted to kairomone-baited Last Call droplets and a sex pheromone baited formulation was almost as attractive to male moths as standard laminate lures.

This work, as well as recent research success with Colorado Potato Beetle, and *Ceratitis* fruit fly formulations, indicates that attracticidal tactics will be important components of future pest management programs.