TEAM NEWSLETTER

Number 13 October 2013

CONTENTS

TO OUR READERS

Marc De Meyer
Chairman of the Steering Committee
marc.de.meyer@africamuseum.be

For those in the northern hemisphere, summer holidays are over. A summer to be remembered with marvelous weather and plenty of sunshine. Other TEAM members will have recovered from their Ramadan festivities (Eid el Fitr) while others are currently celebrating New Year (Rosh Hashana). Different moments to celebrate, mesmerize but also to start working on new research plans or to analyze the data collected over the summer months.

As such we can also think of means and ways to present these results. Therefore, let's keep in mind that the 4-yearly International Symposium on Fruit Flies of Economic Importance is approaching fast. The 9th edition will take place in May 2014 in Bangkok, Thailand but meeting abstract deadline has been postponed. Furthermore, in August 2014, there will also be the 8th International Congress of Dipterology (Potsdam, Germany). The TEAM committee has been looking even further ahead. In the last newsletter we made an appeal for suggestions to organize the next TEAM meeting. The Kolymbari settings and programme will be hard to beat but we had some very attractive offers, so the TEAM steering committee had a hard time making a final selection. The major preference was to have a meeting in Africa, given the fact that the first two took place in Europe (Spain and Crete). In the end, we opted for Stellenbosch, South Africa. Those of you who have been around in the fruit fly research community for a while will certainly remember the ISFEI organized by Brian Barnes and his colleagues in 2002. The same town will now be the setting for the next TEAM meeting, planned to take place in 2016. We are already looking forward to this event and hopefully you will also. Therefore, keep an eye on the next newsletters where we will be providing some more information on this forthcoming meeting.

Announcements with regard to this and other meetings will not only be circulated through the TEAM newsletter but also through the Tephritid Workers Database. Yes, after a long time of hibernation (because of security problems with the former server), the TWD and the related regional group sections (including TEAM) are now up and running again, thanks to Abdeljelil Bakri's relentless efforts to keep TWD going for which we should be very grateful. We have always found the TWD a useful source for information both on recent events or news flashes but also to track down colleagues with expertise in particular fields. Therefore, please take some time to enter or update your profile.

In the mean time, we are providing some interesting literature in this issue.

There is an invited paper by Vicente Navarro-Llopis and Sandra Vacas (Valencia, Spain). They nicely summarize the use of attract and kill devices for fruit fly control. Environmentally friendly devices that can prevent mass spraying on one end, but are also equally effective in reducing or eradicating fruit fly populations, are a major component in fruit fly control programmes. In the last decades, a plethora of different devices and attractants have been designed and promoted. This paper reviews the different approaches in this matter, but also provide some clarity in the terminology associated with this field of research.

Related to the above paper, we also present the synthesis of the doctoral research of Paolo Siciliano, a PhD student Anna Malacrida's lab at the University of Pavia (Italy). Paolo focused his research on the underlying genetic mechanism for pheromone detection and perception in the Medfly as well as the chemical composition of the pheromone cocktail that flies are emitting. Unraveling such mechanisms and components can lead us to understand how pheromones actually work and, in the long term, can assist us in fabricating more efficient attractants to be used in the attract and kill devices discussed in the invited paper.

In addition, Mervyn Mansell (Pretoria, South Africa) informs us on recent fruit fly surveys that have been conducted in a number of African country, thanks to funding of the British Department for International Development, through Trademark, Southern Africa. Mervyn has extensive experience in fruit fly research and especially surveying, through his previous associations with the African Fruit Fly Initiative and USDA-APHIS. For these surveys, intensive use of attractant devices and lures is being made, once again linking up with the other literature in this newsletter. We do hope that you will enjoy the reading of all these inter-related bits.

Finally, as always, we would kindly like to ask you to communicate to us any news or activities that you would like to see included in the next TEAM newsletter.

USE OF ATTRACT AND KILL DEVICES FOR FRUIT FLY CONTROL

The term "attract-and-kill" encompasses all devices that attract target insects to a toxin. We find two different types of devices in this term, traps and bait stations. The main difference between them is that traps retain the flies, whereas bait stations refers only to devices that attract flies to the toxin. Bait stations are defined by Heath et al (2009) as discrete containers of attractants and toxins that attract the pest to the toxin, but in this case, the toxin can kill, sterilize (Navarro-Llopis et al 2007) or infect the target insect (Maniania 2002). In the scientific literature we also find other definitions, such as the term "lure-and-kill" instead of "bait station". El Sayed et al. (2009) define "lure and kill" as a device where "insects are not entrapped at the source of the attractant, but instead the insect is subjected to a killing agent, which eliminates affected individuals from the population after a short period". A classification of "attract and kill" devices and some commercially available examples are depicted in Table 1.

Table 1. Classification and commercially available products of attract and kill.

					Example
Attract and kill	**Mass trapping**	Wet traps	Flies drown in liquid	Liquid baits (protein hydrolysates / ammonium salts)	Ceratrap® Olipe
				Dry attractants + water	Multilure® trap baited with Biolure®+water
		Dry traps	Sticky traps+dry attractant		Jackson trap
			Dry attractant+ insecticide:	Inhalation insecticide (DDVP)	Mosquisan®+ Biolure®
				Contact insecticide (Pyrethroid)	Decis®
	Bait Stations	Lure&Infect			Entomopathogenic traps
		Lure&kill	Contact insecticide (Pyrethroids)		M3®, Vioril® Magnet® MED MAT
			Ingestion insecticide		SPLAT (Anamed®) EPALure&kill®
		Lure&Sterilize			Adress®

With the attract-and-kill technique applying large amounts of toxins to the environment is avoided, as target pests are specifically attracted to the device. This helps greatly reduce the quantity of toxics used and their negative effects on the natural fauna, pesticide residues in fruit, and environmental contamination. The key point for the success of this technique is to have powerful, specific attractants that enable high efficacies to be achieved with the minimum number of devices. In this article, a review of the attractants and devices used for attract-and-kill purposes is provided and the latest trials conducted with these devices are revised.

Past and current devices

There are hundreds of attract-and-kill devices in different countries, including traps and bait stations. Traps have been used since the beginning of the 20th century. Among the first traps used against fruit flies there were glass invaginated devices, known as McPhail traps, which are heavy and fragile. Moreover, the attractants used were liquid, thus making them even heavier and large quantities of water needed to be transported to service all the traps. The most widely used attractants for these first traps were liquid protein baits, fruit juices, soaked rice bran or fermented molasses (Gómez-Clemente 1929). Liquid attractants evaporate and traps become dry in less than 1-2 weeks during warm seasons, or 2 months in colder seasons. Although additional substances were often employed to reduce evaporation the lifespan of liquid attractants seldom exceeded 3 months. One key point in the development of the attract-and-kill technique has been accomplished with the identification of compounds emitted by previously described substances, such as protein baits or fish remains. These substances are food attractants and are, in some cases, attractants per se, but a blend of these substances at the right concentration and adequate release rates enhance attraction significantly. The mixture of ammonium acetate and trimethylamine (Heath et al, 1997 and Heath et al 2004) in the case of *Ceratitis capitata*, the mixture of ammonium salts and putrescine for *Anastrepha* species (Heath et al. 1995, Robacker et al. 1996, Thomas et al. 2008, Holler et al. 2006), or ammonium salts for *Bactrocera oleae* (Broumas and Haniotakis G 1994), were the starting point for the development of new more powerful attractants.

The use of dry attractants based on ammonium salts or amines proved easy to handle and encouraged the development of new device designs. The trap efficacy of these new designs has been tested in several field trials (Epsky et al 1999, Gazit el at 1998, Navarro-Llopis et al 2008) and the results showed that small modifications in the traps can produce substantial increases in efficacy. Overall tephritid flies are attracted to round shapes and colors from yellow to red (from 550 to 680 nm) (*Bactrocera dorsalis,* Wu et al. 2007; *Bactrocera oleae* Katsoyannos and Kouloussis 2001; *Ceratitis capitata* and *Anastrepha fraterculus*, Cytrynowicz et al. 1982). Preferred size is more variable and depends on tephritids species; e.g., *Bactrocera oleae* females prefer small to medium sizes objects of dark colors, whereas *Anastrepha*

ludens females prefer larger objects of yellow color. The results of these studies are often rather controversial demonstrating varying responses to different colors and sizes, with some authors supporting the hypothesis that the contrast of the device against the background is the main visual cue (Brévault and Quilici 2007, Cornelius et al. 1999).

Figure 1. M3® attract and kill device (Biagro, Valencia, Spain). The device contains proteins and vegetable extracts as attractants and α-cypermethrin as a toxicant.

In the last decade, the development of bait stations has intensified rendering them a genuine alternative to mass trapping. The use of new devices, such as the M3® (Biagro, Valencia, Spain) (Picture 1) or Magnet MED® attract and kill device (Suterra LLC, Bend, USA) (Picture 2), increases year after year and mass trapping systems are progressively becoming replaced by bait stations, mainly with the characteristics that will be highlighted in the next two points of this article.

Figure 2. Magnet MED® attract and kill device (Suterra LLC, Bend, USA). This device contains a trimethylamine-ammonium acetate attractant and the surface is coated with deltamethrin as insecticide.

Desirable characteristics of attract-and-kill devices

The use of more powerful attractants has helped reduce the density of devices required to control fruit flies. For example, when a 4% solution of ammonium bi-phosphate was used in Spain for the mass trapping of *Ceratitis capitata*, a density of around 100 traps per ha was employed (Alfaro-Lassala, personal communication). The devices used per se are also essential to obtain good efficacy. By changing only the design of the McPhail-type trap, catches can increase by 3-fold (Navarro-Llopis et al 2008); therefore, using more efficient models can also reduce the density of traps. As a result, the improvement of attractants, from ammonium bi-phosphate to ammonium acetate and trimethylamine, and development of traps with new designs have allowed to reduce the density of required devices from 100 to 40-50 per ha.

Another desirable key attribute for attract-and-kill devices is that they are easy to use. Dry traps and bait stations are preferable to wet devices as liquid management is cumbersome due to weight and spillages. Moreover, devices should be convenient and economic to transport, easy to assemble in the field and inexpensive for the end user (farmers). The transportation of large traps is sometimes costly as they occupy large volumes, and some of them cannot be stacked together because of their design, which results in extra transport costs. Some other models require small parts to be assembled in the field or are cumbersome due to attractant placing, and result in high installation costs. Regarding easiness to assemble and expensiveness for farmers, many new designs, such as the Decis trap® from Bayer Crop Science (Valencia, Spain) (Picture 3) or Cone trap (Probodelt, Spain), have accomplished these characteristics, and we can now find several ready-to-use traps on the market that are fully assembled.

Figure 3. Decis trap® from Bayer Crop Science (Valencia, Spain). The trap contains a food attractant based on ammonium acetate and aliphatic ammines. The trap lid is coated with deltamethrin.

Advantages and disadvantages of attract-and-kill devices

According to Navarro-Llopis et al. (2013), the main advantages of the lure-and-kill systems over mass trapping techniques include: (1) The reduced personnel cost required for their field deployment as lure-and-kill devices are sold preassembled, and no placing of the attractant or trap set-up is needed. (2) The lack of need for an expensive device that retains the flies. In fact, traps are supposed to be retrievable, thus their initial cost should pay off in several years; however, the cost of collecting and washing traps at the end of the season should also be taken into account. (3) Lure-and-kill devices do not became saturated with large number captured insects. This point is especially important when high fruit fly populations are present and relatively small traps are used for mass trapping. (4) Finally, another advantage of the lure-and-kill systems over certain mass trapping devices (dry traps) is that they do not require insecticides to be used as fumigants. The most widely used insecticides in dry traps are dichlorvos (DDVP) and naled. However, these insecticides are now banned in the E.U. and in many countries in the Mediterranean basin, being replaced with contact insecticides, mainly pyrethrins, such as deltamethrin, lambda cyhalothrin or cypermethrin (Alemany et al 2004, Sancho-Sanchez 2009, Tapia-Ramos et al 2012). In some cases, the design of existing traps has been modified to accommodate these contact insecticides.

It can be claimed that a disadvantage of the attract-and-kill technique is its high cost. However, careful calculation of the costs involved suggests that these are comparable to insecticide treatments (Navarro-Llopis et al., 2013). The cost of purchase of devices in Europe is around 150 euros per ha and the cost for their deployment less than 10 euros per ha. On the other hand the cost for the most expensive insecticides (e.g. spinosad) is around 20 euros per ha per treatment, plus 10 euros for the personnel. This treatment should be repeated at least 4 or 5 times, thus bringing the cost involved at similar levels to attract-and-kill devices.

The increasing use of these devices for attract-and-kill purposes has led to a scale economy that has reduced the cost of these devices. In Spain the cost of traps and female attractants for *Ceratitis capitata* has been cut by half in 10 years.

Where to apply

For optimal performance, attract-and-kill systems should be applied in isolated or large areas, so as to reduce fruit fly intrusion from adjacent non-treated areas. It is intuitively obvious that the immigration of pests to a treated area prevents their effective suppression or eradication (Klassen 2005), which is the case of Tephritids given the high mobility of fruit flies. In order to achieve “Area-wide integrated pest management”, trials should be conducted over large or very isolated areas that would affect the whole population of this treated area during a long-term planned campaign (Klassen 2005, Lindquist 2000). This is the ideal situation, but one that cannot always be applied if we take into account the fact that many farmers in the Mediterranean basin own small orchards. For this reason, the application of perimetral trapping to reinforce borders is a successful strategy (Cohen and Yuval, 2000) to implement the attract-and-kill strategy in medium-sized plots (0.5 to 5 ha). The efficacy of his treatment is not clear in plots smaller than 0.5 ha, depending on the type of surrounding crops (presence of hosts), degree of isolation, and even the orientation or direction of the main winds. As a general rule, this methodology is not recommended for small plots where the perimeter represents the main part of the orchard.

Density of devices

Regarding the joint trap plus attractant efficacy effect, the distance between traps can be determined by the effective attraction radius (EAR). The EAR represents the ratio of attractive and passive trap catches and it correlates positively with attractant strength, i.e., distance of attraction. This index is independent of insect density, locality or test duration (Byers et al. 1989). The EAR value for sticky panels baited with the commercial three-component lure (putrescine, ammonium acetate and trimethylamine) was established at 20 m for C. capitata by Peck and McQuate (2000). This result agrees with Leza et al. (2008) or Navarro-Llopis et al. (2013),

who demonstrated that 50 mass trapping or attract-and-kill devices baited with ammonium acetate and trimethylamine dry dispensers prove more efficient to significantly reduce fruit damage at similar levels as weekly applications of insecticide. However, when using protein baits, the number of traps required was higher, as many as 200 traps per hectare are required (Alemany et al. 2004). In any case, recommended density did not guarantee the total protection of fruit against fruit fly, but the fly population and fruit damage reduced and only some support insecticide treatments were required.

The efficacy of combinations of traps and lures has been studied recently for economically important fruit flies: *C. capitata* (Gazit et al. 1998, Navarro-Llopis et al. 2008), *Anastrepha* spp. (Diaz-Fleischer et al. 2009), B. tryoni (Dominiak and Helen 2010), *B. dorsalis* (Cornelius et al. 2000), and *B. oleae* (Broumas et al. 1994).

Application time

The main aim of the attract-and-kill technique is to suppress the fruit fly populations; the longer attractants are active in the field the greater the achievable suppression of a particular device. Replacement of attractants may be expensive but it might be necessary in some cases in order to cover a wider window of time to adequately control fruit fly populations. Wet traps using protein-based attractants or ammonium salts are the most sensitive to weather conditions and monthly replacement of the baits tends to be required. In contrast, dry attractants have longer life spans and can be used for as long as 4-6 months (Colas et al. 2012, Navarro-Llopis et al. 2013). For the Mediterranean fruit fly in the Mediterranean basin, mass trapping or bait stations are placed in the field at least one generation before fruit start ripening and they remain active for until 4 months later when harvesting is completed. Therefore, for *C. capitata* attractants that last 4 months are required for an efficient mass-trapping strategy.

Efficacy

Efficacy assessments should be conducted to measure both fruit fly population reduction and fruit damage. However, many works in which fruit fly population reduction has been reported either do not include such data at all or include rather poor data. Nevertheless, in several recent works the attract-and-kill technique has proven as effective as insecticide sprays against *C. capitata* in citrus orchards (Ben Jemaa et al.2010, Leza et al 2008, Navarro-Llopis et al 2013), or against the olive fruit fly (Haniotakis et al 1991, Broumas 2002).

As previously mentioned, in contrast to the application of insecticides this methodology does not have an instant effect on the population. Therefore, monitoring fruit fly populations should be conducted when the attract-and-kill technique is applied in the field. Monitoring will help farmers to decide if a support insecticide treatment is required. Whether such a treatment is required depends on the sensitivity of the crop to fruit fly puncture and the ripening status. For example in Spanish citrus orchards, the upper threshold for insecticide treatment is 1 female per trap/day in a McPhail-type trap with an attractant mixture of ammonium acetate, trimethylamine and putrescine. This threshold has been established after more than 10 years of field trials being conducted in citrus crops, but it is completely empirical. No fruit damage over 1% is expected if the fruit fly population is maintained below this threshold during ripening. However this threshold should be establish for each crop, with each combination of trap and attractant.

As a concluding remark, the use of the attract-and-kill methods is increasing year after year as they are becoming affordable for growers and their efficacy has vastly improved. The development of new female attractants for other fruit fly species will expand the use of this pest management method. Although the attract-and-kill technique by itself is not always adequate to control a pest, the population reduction achieved enables the use of the sterile insect technique (SIT) in areas with high populations in which control is barely manageable by this method. Area-wide strategies that combine the use of attract-and-kill technique with SIT, timely application of insecticides and biological control agents are expected to obtain the best fruit fly control results.

References

Alemany A, Miranda MA, Alonso R et al (2004) Efficacy of *C. capitata* (Wied) (Diptera: Tephritidae) female mass trapping. Edge-effect and pest multiplier role of unmanaged fruit host. Bol San Veg Plagas 30: 255-264

Alemany A, Miranda MA, Fransen TV and Abdali A (2007). Deltamethrine as a replacement for diclorvos (DDVP) strips used as a retention system in fruit fly traps. Development of Improved Attractants and Their Integration into Fruit Fly SIT Management Programmes, 11

Ben Jemaa JM, Bachrouch O, Allimi E and Dhouibim MH, Field evaluation of Mediterranean fruit fly mass trapping with Tripack (R) as alternative to malathion bait-spraying in citrus orchards. Spanish J Agric Res 8: 400-408 (2010).

Brévault T. and Quilici S (2007), Visual response of the tomato fruit fly, *Neoceratitis cyanescens*, to colored fruit models. Entomol Exp Appl 125: 45-54

Broumas T, Haniotakis G (1994) Comparative field studies of various traps and attractants of the olive fruit fly, *Bactrocera oleae*. Entomol Exp Appl 73: 145-150

Broumas T, Haniotakis G, Liaropoulos C et al (2002) The efficacy of improved form of the mass-trapping method for the control of the olive fruit fly, Bactrocea oleae (Gmelin) (Dipt., Tephritidae): pilot-scale feasibility studies. J Appl Entomol 126: 217-223

Byers JA, Anderbrant O, Löqvist J (1989) Effective attraction radius. J Chem Ecol 15: 749-765

Cohen H, Yuval B (2000) Perimeter trapping strategy to reduce Mediterranean fruit fly (Diptera: Tephritidae) damage on different host species in Israel. J Econ Entomol 93: 721-725

Colas C, Domínguez M, Martí S et al (2012) Magnet MED: A new long-life, ready to use "Attract and kill" system to control Mediterranean fruit fly Ceratitis capitata Wiedemann. In: Book of Abstracts of the XII International Citrus Congress. November 2012, Valencia, Spain, p 229

Cornelius ML, Duan JJ, Messing RH (1999) Visual stimuli and the response of female oriental fruit flies (Diptera: Tephritidae) to fruit mimicking traps. J Econ Entomol 92: 121–129

Cornelius ML, Nergel L, Duan JJ et al (2000) Responses of female oriental fruit flies (Diptera: Tephritidae) to protein and host fruit odors in field cage and open field tests. Environ Entomol 29: 14-19

Cytrynowicz M, Morgante JS and de Souza HM (1982) Visual responses of South American fruit flies, Anastrepha fraterculus, and Mediterranean fruit flies, Ceratitis capitata, to colored rectangles and spheres. Environ Entomol, 11(6), 1202-1210.

Diaz-Fleischer F, Arredondo J, Flores S et al (2009) There is no magic fruit fly trap: multiple biological factors influence the response of adult Anastrepha ludens and Anastrepha oblique (Diptera: Tephritidae) individuals to MultiLure traps baited with BioLure or NuLure. J Econ Entomol 102: 86-94

Dominiak BC, Helen N (2010) Field performance of Lynfield and McPhail traps for monitoring male and female sterile Bactrocera tryoni (Froggatt) and wild Dacus newmani (Perkins). Pest Manag Sci 66: 741-744

El-Sayed AM, Suckling DM, Byers JA et al (2009) Potential of "lure and kill" in long-term pest management and eradication of invasive species. J Econ Entomol 102: 815-835

Epsky ND, Hendrichs J, Katsoyannos BI et al (1999) Field evaluation of female-targeted trapping systems for Ceratitis capitata (Diptera: Tephritidae) in seven countries. J Econ Entomol 92: 156-164

Gazit Y, Rössler Y, Epsky ND et al (1998) Trapping females of the Mediterranean fruit fly (Diptera: Tephritidae) in Israel: comparison of lures and trap type. J Econ Entomol 91: 1335-1359

Gómez Clemente F (1929) Experiencias de lucha contra la Ceratitis capitata Wied. con cazamoscas de vidrio. Bol Pat Veg Ent Agr 4:21-38

Haniotakis G, Kozyrakis M, Fitsakis T et al (1991) An effective mass trapping method for the control of Dacus oleae (Diptera: Tephritidae). J Econ Entomol 84: 564-569

Heath RR, Epsky ND, Guzman A et al (1995) Development of a dry plastic insect trap with food-based synthetic attractant for the Mediterranean and the Mexican fruit flies (Diptera: Tephritidae). J Econ Entomol 88: 1307-1315

Heath RR, Epsky ND, Dueben BD et al (1997) Adding methyl-substituted ammonia derivatives to a food-based synthetic attractant on capture of Mediterranean and Mexican fruit flies (Diptera: Tephritidae). J Econ Entomol 90: 1584-1589

Heath RR, Epsky ND, Midgarden D et al (2004) Efficacy of 1,4-diaminobutane (putrescine) in a food-based synthetic attractant for capture of Mediterranean and Mexican fruit flies (Diptera: Tephritidae). J Econ Entomol 97: 1126-1131

Heath RR, Lavallee SG, Schnell E, et al (2009), Laboratory and field cage studies on female-targeted attract-and-kill bait stations for Anastrepha suspensa (Diptera: Tephritidae). Pest Manag Sci 65: 672-677.

Holler T, Sivinski J, Jenkins C et al (2006) A comparison of yeast hydrolysate and synthetic food attractants for capture of Anastrepha suspensa (Diptera: Tephritidae) Fla Entomol 89: 419-420

Katsoyannos BI, Kouloussis NA (2001) Captures of the olive fruit fly Bactrocera oleae on spheres of different colours. Entomol Exp Appl 100:165-172

Klassen W. Area-Wide Integrated Pest Management and SIT, in Sterile Insect Technique. Principles and Practice in Area-Wide Integrated Pest Management, ed. by Dyck VA, Hendrichs JH and Robinson AS. Springer, Dordrecht, The Netherlands, pp. 39-68 (2005).

Leza MM, Juan A, Capllonch M and Alemany A, Female-biased mass trapping vs. bait application techniques against the Mediterranean fruit fly, Ceratitis capitata (Diptera: Tephritidae). J Appl Entomol 132: 753-761 (2008)

Lindquist DA, Pest management strategies: area-wide and conventional, in FAO/IAEA International Conference on Area Wide Control of Insect Pests - International Symposium on Fruit Flies of Economic Importance, ed by Penerbit Univ. Sains Malaysia, Pulau, Penang, Malaysia (2000)

Maniania, N.K. (2002) A Low-cost Contamination Device for Infecting Adult Tsetse Flies, Glossina spp., with the Entomopathogenic Fungus Metarhizium anisopliae in the Field. 12(1) 66: 511-519

Navarro-Llopis V, Alfaro F, Domínguez J et al (2008) Evaluation of traps and lures for mass trapping of Mediterranean fruit fly in citrus groves. J Econ Entomol 101: 126-131

Navarro-Llopis V, Domínguez-Ruiz J, Zarzo M et al (2010). Mediterranean fruit fly suppression using chemosterilants for area-wide integrated pest management. Pest Manag Sci, 66(5): 511-519.

Navarro-Llopis V, Pimo J, and Vacas S. (2013). Efficacy of attract-and-kill devices for the control of *Ceratitis capitata*. Pest Manag Sci 69(4): 478-482.

Peck SL, McQuate GT (2000) Field tests of environmentally friendly malathion replacements to suppress wild Mediterranean fruit fly (Diptera: *Tephritidae*) populations. J Econ Entomol 93: 280-289

Robacker DC, Moreno DS, Demilo AB (1996) Attractiveness to Mexican fruit flies of combinations of acetic acid with ammonium/amino attractants with emphasis on effects of hunger. J Chem Ecol 22: 499-511

Sancho-Sánchez J (2009) Killdisc: una alternativa para asegurar la viabilidad futura de la técnica de captura masiva contra mosca de la fruta. Phytoma 213: 56-57

Tapia-Ramos E, Acin P, Casas JI (2012) Decis® Trap: a new biorational tool to manage populations of *Ceratitis capitata* (Diptera: *Tephritidae*). In: Proceedings of the 2nd International Symposium of TEAM. Kolymbari, Greece. Ziti Publications, Thessaloniki, Greece, p 100

Thomas DB, Epsky ND, Serra CA et al (2008) Ammonia formulations and capture of Anastrepha fruit flies (Diptera: *Tephritidae*). J Entomol Sci 43: 76-85

Vicente Navarro-Llopis and Sandra Vacas
Instituto Agroforestal del Mediterráneo-Universidad Politécnica de Valencia. Camino de Vera s/n. Edificio 6C. 5ª planta. 46022. Valencia, Spain.
vinallo@ceqa.upv.es

UNRAVELLING MOLECULAR COMPONENTS OF PHEROMONE PERCEPTION IN THE MEDFLY, *CERATITIS CAPITATA*

PAOLO SICILIANO
Dissertation for a Ph.D. in Cellular Biology,
Department of Biology and Biotechnology,
University of Pavia (Italy) 2012

Chemoreception plays an important role in insect intra- and inter-sex communication, inducing specific behavioural responses in terms of sexual attraction, mating aggregation and host-marking of oviposition sites. The perception of chemical signals relies primarily on olfaction, which mediates the recognition of volatile signals, and gustation which permits the discrimination of soluble stimulants (Sanchez-Gracia et al. 2009). Chemoreception is facilitated by a signal transduction cascade involving odorant-binding proteins (OBPs) (Voght and Riddiford 1981), chemosensory proteins (CSPs) (Pelosi et al. 2006), and olfactory, gustatory and ionotropic receptors (ORs, GRs and IRs, respectively) (Leal 2013). OBPs are small, globular, highly abundant water-soluble proteins secreted into the sensilla lymph by non-neuronal auxiliary cells. These proteins bind and solubilize odours that enter the pores in the sensilla, and transport them through the aqueous lymph to activate the membrane bound ORs (Leal 2013; Vieira and Rozas 2011). Within the OBP family, pheromone-binding proteins (PBPs) are regarded as transporters of sex pheromone components. The molecular machinery underlying these processes is still unknown and is the target of numerous studies.

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann; Diptera: Tephritidae) shows an extraordinarily diverse larval host range (Malacrida et al. 2007), which in turn is reflected in its impressive biological success. Its invasive potential can, in part, be ascribed to the ability of its olfactory systems to i) localise different plant hosts, ii) detect pheromones during the recognition and location of mates, and iii) to discriminate between suitable and already pierced hosts for oviposition.

Information on the olfactory mechanisms of the medfly can furnish useful tools for the implementation of pest control programmes and, in the long term, permit the use of pheromone components and other volatile compounds as targets for the development of synthetic attractants/repellents.

On this basis, this PhD project was directed at:

1. The identification of genes putatively involved in pheromone perception (Pbps);

2. The expression profile analyses of the identified putative Pbp genes, in relation to sex, tissue, age and physiological status;

3. The purification of the chemicals emitted by both sexes during the courtship in order to identify the components of the pheromone blend and those that are electro-physiologically active;

4. The heterologous expression and subsequent purification of the identified putative PBPs for ligand-protein binding studies.

A screening of the three EST libraries derived from embryos, heads and testes/accessory glands (Gomulski et al. 2008, 2012; Scolari et al. 2012) allowed the identification of a first set of sequences (Ccpbp1-Ccpbp5) with very high similarity to D. melanogaster Pheromone binding protein related protein (Pbprp) genes.

Molecular analyses on these five medfly transcripts enabled the assessment of the presence of all the features of the PBP family, i.e.: i) a putative signal peptide; ii) a hydrophobic domain, and iii) the six conserved cysteine residues. Comparative structural analyses were used to help elucidate the function of the putative medfly PBPs. Alignments performed using PHYRE (Kelley and Sternberg 2009) enabled the prediction of the hypothetical structure of each putative medfly PBP by comparison with the real structures of similar proteins in the databases. All the structures, visualized using PYMOL (Delano 2002), showed a globular shape and the presence of six alpha-helixes and three disulphide bridges in highly conserved positions (Figure 1).

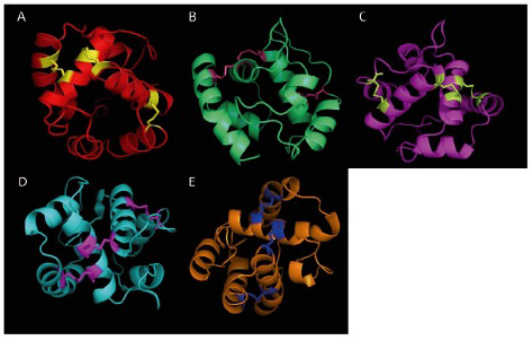

Figure 1. Three-dimensional models of the five medfly predicted PBPs. (A) CcPBP1, (B) CcPBP2, (C) CcPBP3, (D) CcPBP4, (E) CcPBP5. In each hypothetical structure, three highly conserved disulphide bridges and six alpha-helixes are represented.

The expression profiles in different tissues of the five *Ccpbp* genes resulted in predominant transcript abundance in the main olfactory tissues, namely antennae and maxillary palps. *Ccpbp5* displays high expression also in the tarsi, which have a role in gustation. Interestingly, *Ccpbp4* has a clear antenna-specific expression. The expression of all five putative Pbp genes in both sexes is congruent with the reproductive behaviour of the species. In the medfly, males aggregate in small groups (leks) from where they start to display a complex "calling" courtship behaviour (Yuval and Hendrichs 2000) that attracts females to the lekking sites. Thus, males need first to recruit other males to form the lek and only later the females perceive the pheromone signals emitted by the males. Thus, both sexes need to express proteins capable of recognising these chemical compounds (Figure 2).

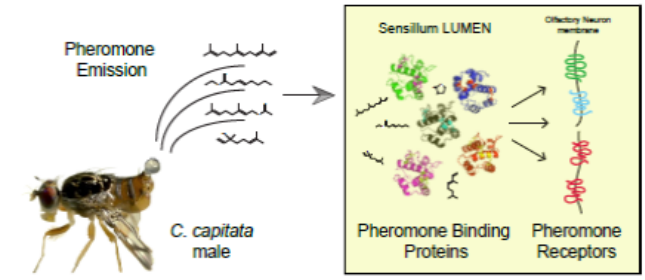

Figure 2. Medfly male with expanded vesicle-like rectal ampulla, protruding from the erected anal papilla.

Out of the five candidates, *Ccpbp4* was chosen as the best candidate for protein purification and relative binding studies aimed at the identification of binding specificity to one or more of the medfly emitted chemicals involved in inter and intra-sex communication.

Different approaches and techniques have previously been applied to isolate and purify compounds emitted by medfly individuals during "calling" (Jang et al. 1989; Flath et al. 1993; Goncalves et al. 2006; Alfaro et al., 2011). The project used the ISPRA strain, the same as that used for the on going medfly Genome sequencing project.

As expected, males emit a complex mixture of chemicals, whereas no chemical emission was detected in the female counterpart, under the same experimental conditions. The collected mixture was tested on GC-EAG (*gas chromatography* coupled with electroantennogram recording), which showed that 19 compounds were able to elicit an electrophysiological response on female antennae, while only 14 compounds elicited responses in the males (Siciliano et al. in preparation). This preliminary data, taken together with the presence of a signal peptide at the N' terminal, six cysteines in highly conserved positions, antennal specific and sexual maturation-related gene expression, an hypothetical peptide of about 150 amino acids, a predicted globular shaped related protein with 6 α-helixes, make *Ccpbp4* a good pheromone binding protein gene candidate. Binding assays are now in progress for the other putative medfly PBPs (CcPBP1, CcPBP2, CcPBP3 and CcPBP5).

These results contribute to the clarification of the olfactory mechanisms in the Mediterranean fruit fly, and will permit the identification of differences and similarities among tephritid species. The medfly could become a model for the study of olfaction in this family and the identification of the main actors in the pheromone blend, together with their specific binding proteins and receptors could furnish useful tools for the improvement of current pest management control techniques applied worldwide.

References

Alfaro C, Vacas S, Zarzo M, Navarro-Llopis V, Primo J (2011) Solid phase microextraction of volatile emissions of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae): influence of fly sex, age, and mating status. J Agric Food Chem 59: 298-306.

DeLano WL (2002) The PyMOL Molecular Graphics System (http://www.pymol.org).

Flath RA, Jang EB, Light DM, Mon RT, Carvalho L, Binder RG, John JO (1993) Volatile pheromonal emissions from the male Mediterranean fruit fly: effects of fly age and time of day. J Agric Food Chem 41:830-837.

Gomulski LM, Dimopoulos G, Xi Z, Scolari F, Gabrieli P, Siciliano P, Clarke AR, Malacrida AR, Gasperi G (2012) Transcriptome profiling of sexual maturation and mating in the Mediterranean fruit fly, *Ceratitis capitata*. PLoS ONE 7: e30857.

Gomulski LM, Dimopoulos G, Xi Z, Soares MB, Bonaldo MF, Malacrida AR, Gasperi G (2008) Gene discovery in an invasive tephritid model pest species, the Mediterranean fruit fly, *Ceratitis capitata*. BMC Genomics 9:243-257.

Goncalves GB, Silva CE, Dos Santos JCG, Dos Santos ES, Do Nascimento RR, Da Silva EL, Mendonca ADL, De Freitas MRT, Sant'Ana AEG (2006) Comparison of the volatile components released by calling males of *Ceratitis capitata* (Diptera: Tephritidae) with those extractable from the salivary glands. Fla Entomol 89: 375-379.

Jang EB, Light DM, Binder RG, Flath RA, Carvalho LA (1989) Electroantennogram response of the Mediterranean fruit fly, *Ceratitis capitata*, to

identified constituents from calling males. Entomol Exp Appl 50: 7-19.
Kelley LA, Sternberg MJE (2009) Protein structure prediction on the web: a case study using the Phyre server. Nature Protocols 4: 363-371.
Leal WS (2013) Odorant reception in insects: roles of receptors, binding proteins, and degrading enzymes. Annu Rev Entomol 58: 373-391.
Malacrida AR, Gomulski LM, Bonizzoni M, Bertin S, Gasperi G, Guglielmino CR (2007) Globalization and fruitfly invasion and expansion: the medfly paradigm. Genetica 131: 1-9.
Pelosi P, Zhou JJ, Ban LP, Calvello M (2006) Soluble proteins in insect chemical communication. Cell Mol Life Sci 63: 1658-1676.
Sanchez-Gracia A, Vieira FG, Rozas J (2009) Molecular evolution of the major chemosensory gene families in insects. Heredity (Edinb) 103: 208-216.
Scolari F, Gomulski LM, Ribeiro JM, Siciliano P, Meraldi A, Falchetto M, Bonomi A, Manni M, Gabrieli P, Malovini A, Bellazzi R, Aksoy S, Gasperi G, Malacrida AR (2012) Transcriptional profiles of mating-responsive genes from testes and male accessory glands of the Mediterranean fruit fly, *Ceratitis capitata*. PLoS ONE 7: e46812.
Vieira FG, Rozas J (2011) Comparative genomics of the odorant-binding and chemosensory protein gene families across the Arthropoda: origin and evolutionary history of the chemosensory system. Genome Biol Evol 3: 476-490.
Vogt RG, Riddiford LM (1981) Pheromone binding and inactivation by moth antennae. Nature 293: 161-163.
Yuval B, Hendrichs J (2000) Behavior of flies in the genus *Ceratitis* (Dacinae: Ceratitidini). In: Aluja M, Norrbom A (eds) Fruit flies (Tephritidae): phylogeny and evolution of behavior. CRC Press, Boca Raton, Fla, pp 429-457.

PEOPLE: PAOLO SICILIANO

Paolo Siciliano initiated his PhD studies in October 2008 under the supervision of Professor Anna Rodolfa Malacrida, at the Laboratory of Insect Evolutionary Molecular Biology, Department of Biology and Biotechnology, University of Pavia (Italy). On February 2012, he successfully defended his PhD Thesis entitled "Unravelling sex communication in flies: identification and characterisation of genes, proteins and chemicals involved in pheromone perception in the severe pest species *Ceratitis capitata* (medfly)".

During his PhD, he worked on a project aimed at acquiring knowledge and disentangle the components of the molecular machinery which mediates odour and pheromone perception in *Ceratitis capitata* (medfly). The long term goal is the identification of potential useful targets/tools to implement the current control strategies against this pest.

Dr. Siciliano's research was supported by funding from the Italian Ministry of Education, University and Research (PRIN 20077RCHRW, awarded to Dr. Ludvik M. Gomulski). During the course of his PhD studies, Dr. Siciliano collaborated with the Department of Biological Chemistry, Rothamsted Research, Harpenden (United Kingdom), and with the Institute of Organic Chemistry and Biochemistry, ASCR Prague (Czech Republic).

Following his PhD studies, Dr. Siciliano worked as Senior Scientist at Oxitec Limited (Abingdon, Oxfordshire, United Kingdom).

Dr. Siciliano is currently working as a Senior Research Scientist and Project Manager at Inscentinel Limited (Harpenden, Hertfordshire, United Kingdom).

Dr. Paolo Siciliano
Inscentinel Ltd, Rothamsted Research Harpenden,
Hertfordshire AL5 2JQ
United Kingdom.
paolo.siciliano@unipv.it

RESEARCH SCIENTIST POSITION

Oxitec Limited, UK
Permanent position

Purpose of job: To join a small dynamic team, in a friendly bio-tech company (SME), investigating new and exciting technologies to control a range of insects that are important agricultural and public health pests. Oxitec is developing genetic technology that will make the environmentally friendly sterile insect technique more cost-effective and more widely applicable (www.oxitec.com).

Your job will be helping to develop the sterile insect technique and will involve:

- Rearing and maintenance of insect colonies.
- Insect genetics and fluorescent marker analysis.
- A range of molecular biology techniques (PCR, RT-PCR, genomic DNA and RNA extractions).
- Genetic transformation of a range of insect species.

Training in the necessary molecular, genetic and entomological methods will be provided.

Attributes of the successful candidate will include:

- A degree level (BSc, MSc or recent PhD) in a relevant scientific area
- Laboratory experience of rearing insects and/or molecular biology would be desirable.
- Being able to work independently and within a team.
- Good communication skills and a diligent attitude.

The successful applicant should show enthusiasm to work with insects and a keen nature to learn and develop their skills within the company. Only applicants available for fast start need apply.

Starting salary in the range £20,000 – £28,000, depending on experience.

Contact: Please apply in writing:
totracy.thompson@oxitec.com including your CV.

AFRICAN FRUIT FLY SURVEYS

Formal comprehensive surveys for fruit flies in Africa commenced in the early 2000's with the launch of the African Fruit Fly Initiative (AFFI) at the International Centre for Insect Physiology and Ecology (ICIPE) in Nairobi, Kenya. This led to the initial detection of the invasive *Bactrocera invadens* in Africa in 2003. The ICIPE initiative covered mainly Kenya, Tanzania and Uganda. In 2004 the Animal and Plant Health Inspection Services of the United States Department of Agriculture (USDA-APHIS), Pretoria, South Africa, launched an assistance programme to enable countries to set up surveillance programmes and to provide technical backup to other countries, to monitor the threat of *B. invadens, B. cucurbitae* and other economically important fruit flies. USDA-APHIS initiated surveys in Angola, Botswana, Ethiopia, Malawi, Mozambique, Namibia, Rwanda, Swaziland and Zambia, while the South African NPPO conducted its own surveys, together with the private sector. The objective of the USDA-APHIS programme was to provide traps and lures, and training courses in fruit fly taxonomy, economic impact, management and trapping protocol. It also provided an identification service to all countries as part of the support programme.

Mervyn Mansell (centre) and colleagues preparing fruit fly traps for an USDA-APHIS survey in Mozambique, when assessing the geographical distribution of *Bactrocera invadens.*

Additional funding for African fruit fly activities has subsequently been provided through Trademark, Southern Africa (TMSA), an implementation agency for the British Department for International Development (DFID). This funding has enabled countries to support far more extensive fruit fly programmes that now include

management, as well as detection, delimitation and monitoring surveillance programmes and training. The funding has included vehicles, chemicals for surveillance and management, procurement and provision of equipment, as training courses in taxonomy, economic impact, surveillance protocols, and management, record-keeping and reporting. Surveillance covers all economically important species in the African genera *Ceratitis*, *Dacus* and *Bactrocera*, to enable countries to develop comprehensive fruit fly pest lists. It is also aimed at enabling countries to become self-sufficient in fruit fly surveillance, management and control. To date, Malawi, Swaziland and Zimbabwe have benefited from the TMSA-DFID funding model, with negotiations in progress to assist Botswana and Mozambique in a similar manner. The initial hands-on trap deployment training, as well as formal training, as well as the identification service has been provided by the TMSA fruit fly consultant, Dr Mervyn Mansell, University of Pretoria, who was also responsible for the initiation of the USDA-APHIS programme.

Mervyn W. Mansell
Department of Zoology and Entomology,
University of Pretoria,
Private Bag X20, Hatfield, 0028 Pretoria,
South Africa.

ERRATUM

An article of the previous issue of our newsletter (No. 12, December 2012) contains the incorrect information (page 16) that during the 2nd International TEAM Meeting in Crete a poster award was given to Amani Mohamed Khair Abbas (Shendi, Sudan).

The correct information is that the poster awards were given to Domingos Raquene Cugala (Maputo, Mozambique), Faiza Elgaili Elhassan Salah (Wad Medani, Sudan) and Mitra Moezipour (Karaj, Iran).

FORTHCOMING MEETINGS

8th INTERNATIONAL CONGRESS OF DIPTEROLOGY
10-15 August 2014
Potsdam, Germany
http://www.icd8.org

9th INTERNATIONAL SYMPOSIUM ON FRUIT FLIES OF ECONOMIC IMPORTANCE
20-25 April 2014
Bangkok, Thailand
malavasi@moscamed.org.br

XXV INTERNATIONAL CONGRESS OF ENTOMOLOGY (ICE 2016)
25-30 September 2016
Orlando, Florida, USA
http://www.ice2016orlando.org/

THIS NEWSLETTER

This newsletter is intended for the publication of subjects of interest to the members of TEAM. All content is solicited from the membership and should be addressed to the members of the editorial board.