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WORKING MATERIAL

Assessment of Simultaneous Application of SIT and MAT to Enhance Management of **Bactrocera** *and* **Zeugodacus** *Fruit Flies*

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CRP Summary

The male annihilation technique (MAT) is an environmentally benign pest control method. MAT, which lures and kills the male insects, has been used to suppress certain fruit fly pest species in the tribe Dacini (including the genera *Bactrocera* and *Zeugodacus*) as part of an integrated pest management approach. In some cases, it has even been successfully applied to eradicate insect populations on isolated islands or in outbreak situations. The sterile insect technique (SIT), which is a target-specific technique with no known negative environmental impacts, involves the mass-rearing of male insects, sterilising them with ionising radiation, and releasing them in the target area in numbers large enough to outcompete their wild counterparts. Copulations of sterile male insects with wild female insects result in no offspring. In certain cases, the resultant level of population suppression can lead to eventual eradication of the target population. Furthermore, as SIT acts in an inverse density dependent manner, it becomes more effective when the wild population is reduced. Integration of MAT with SIT has so far been sequential, rather than simultaneous, with SIT applied after a significant reduction of the wild population with MAT. This was to avoid the mass-trapping or killing of released sterile males by MAT devices baited with semiochemicals such methyl eugenol (ME) or cuelure (CL). It was assumed that attraction of sterile males to traps or MAT devices would significantly reduce the efficacy of SIT.

The development of cost-effective semiochemical treatments and delivery systems that improve sterile male performance and reduce their response to semiochemicals is highly desirable so that MAT and SIT could be integrated. Even though the incorporation of semiochemical treatments/supplements appears feasible, practical and standard procedures of implementation at an operational scale still need to be developed and validated. Thus, exploring the potential of such approaches is essential in view that the simultaneous MAT and SIT application has considerable potential to improve sterile to wild male overflooding ratios and therefore SIT cost-effectiveness. The combination of male replacement plus male enhanced performance increases what can be achieved with the same number of sterile flies: treating a wider area or enabling more rapid suppression or eradication. Furthermore, due to the increased cost-effectiveness, decisions to invest in SIT may be facilitated in situations where this would not otherwise be feasible.

The CRP objective is to explore the potentially synergistic relationship between MAT and SIT when applied simultaneously to improve the efficacy of *Bactrocera* and *Zeugodacus* fruit fly management. The assessment of semiochemicals to enhance SIT application against these pest fruit flies will include:

1. Assessment of the effect of exposure of major dacine pest species to semiochemicals on earlier sexual maturation and improved male sexual performance, as well as reduced response of semiochemical-exposed sterile males to MAT,
2. Evaluation of key parameters in semi-field cages such as degree of lure response of sterile flies, sterile:wild over-flooding ratio and bisexual release to determine their influence on the effectiveness of simultaneous MAT and SIT, and
3. Semi-field and field evaluation of simultaneous MAT and SIT within a pilot or operational setting that includes compatible management practices.

1. Background

The sterile insect technique (SIT) requires the mass production of insects, which takes place in specially designed rearing facilities where insects are adapted to indoor conditions (Klassen, 2005). The mass-reared insects are irradiated at the late pupal stage and transported to emergence and release facilities. They are subsequently placed in containers for adult emergence and feeding before being released to target field areas. Prior to release, there is potential to manipulate sterile males in a manner that will significantly improve their mating performance by pre-release treatments such as semiochemical treatments and provision of semiochemical supplements (Akter et al., 2017a; Haq & Hendrichs, 2013).

The SIT is more efficient when integrated with other suppression methods. So far, the integration of SIT with male annihilation technique (MAT) has been implemented only sequentially for fruit flies in the tribe Dacini (including the genera *Bactrocera* and *Zeugodacus*). The MAT is used first to reduce the wild male population (Vargas et al., 2014), followed by the release of sterile males after the removal of MAT devices. However, modeling suggests the simultaneous use of both methods could significantly increase the effectiveness of fruit fly suppression and eradication (Barclay et al., 2014).

The simultaneous use of MAT and SIT may be compromised by elimination of sterile males via MAT. However, studies of multiple *Bactrocera* and *Zeugodacus* species show that pre-release exposure of sterile males to semiochemicals reduces their response to lures formulations in MAT (Akter et al., 2017a; Shelly, 1994). This results in "male replacement": sterile males will remain in the field while their wild counterparts are removed by MAT. Furthermore, it has been shown that feeding on semiochemicals [e.g., methyl eugenol (ME), raspberry ketone (RK)] significantly increases male courtship activity, improves male mating success, and accelerates sexual development of some *Bactrocera* species (Hee & Tan, 1998; Shelly et al., 2010; Tan & Nishida, 2000; Wee et al., 2007).

The development of cost-effective semiochemical treatments that improve sterile male sexual performance and reduce their response to MAT is highly desirable. Even though the incorporation of semiochemical supplements for *Bactrocera* and *Zeugodacus* species appears feasible, practical procedures for their implementation at an operational scale still need to be developed. Nevertheless, exploring the potential of such approaches appears essential in view that the simultaneous application of MAT and SIT has considerable potential to drastically increase sterile to wild male overflooding ratios and therefore SIT cost-effectiveness (Barclay et al., 2014). The combination of male replacement plus male enhanced performance increases what can be achieved with the same number of sterile flies: treating a wider area or enabling more rapid eradication. Furthermore, due to the increased cost-effectiveness, decisions to invest in SIT may be facilitated in situations where this would not otherwise be feasible.

1.1.Scenarios

The potentially synergistic relationship between MAT and SIT when applied simultaneously can dramatically improve the efficacy of fruit fly management. It would be particularly desirable for those species where the effectiveness of MAT is limited, but also has great potential for *Bactrocera* and *Zeugodacus* fruit fly control programmes (Table 1).

The current literature suggests that ME is a much stronger lure than CL (Vargas et al., 2014). For species that respond strongly to ME, including *B. dorsalis* and *B. correcta*, one may be able to rely on MAT alone to eradicate incursions and introductions, but not established populations. Eradication of incursions and introductions of other ME-responding species that are not as strongly attracted by the lure (e.g., *B. carambolae*), would likely benefit from the simultaneous application of MAT and SIT. Similarly, eradication of incursions and introductions of CL-responding species would benefit greatly from the simultaneous application of MAT and SIT.

The expected benefits of simultaneous MAT and SIT could make it a good option for treating established populations of species responsive to either ME or CL. In many instances it may not be technically feasible to add SIT to MAT because mass-rearing facilities are not available for the target species, or there are insufficient resources to handle and release sterile insects. However, sterile flies could be sourced from existing mass-rearing facilities in other countries.

Table 1. Scenarios under which simultaneous MAT and SIT application is likely to improve fruit fly control. ME = methyl eugenol; CL = cuelure

Population status*	ME responsive^	CL responsive
Incursion (Isolated recent detection)	MAT	MAT + SIT
Introduction (Small breeding population)	MAT†	MAT + SIT
Established	MAT + SIT	MAT + SIT

* See complete definitions in ISPM 5 (2019).
^ Differential response of ME-responding species to the lure (e.g., *B. carambolae* exhibits a weaker response that *B. dorsalis*) would also influence if simultaneous MAT and SIT should be used to control incipient populations.
†SIT may be considered

1.2.Main assumptions

The participants of the CRP came with the following assumptions to set up and organize the working plan for the entire duration of the CRP:

1. Exposure of some *Bactrocera* and *Zeugodacus* species to semiochemicals leads to earlier sexual maturation and significant improvement of male sexual performance. In addition, there is a 70% reduction in trapping of males of some species that have been exposed to a semiochemical before release. Attempts need to be made to increase the percentage of non-responding males while also maintaining the benefits of pre-release treatment. This work can be performed in the laboratory or in field cages. Specific objectives to achieve this goal are to:
    a. Determine the minimum amount of semiochemical required by males to reduce their lure response for a significant portion of their lifetime in the field

b. Establish the best means to confirm that adequate semiochemical delivery has been achieved physiologically, including analysis of haemolymph, rectal gland contents, and pheromone composition
c. Establish the best means to minimise responsiveness to traps
d. Identify alternative semiochemicals for pre-release treatment, but recognising that the best lure for a particular fruit fly species may not be the best semiochemical for pre-release treatment
e. Compare the mating age and behaviour of males fed semiochemicals only or in combination with other pre-release treatments (e.g., methoprene, dietary supplements)
f. Determine whether pre-release treatments of fruit flies diminish their performance, such as survival, flight, dispersal, and mating ability
g. Determine the best means of semiochemical delivery that is compatible with existing fly emergence and release systems
h. Establish the relative field response of different fruit fly species to male lures using standardised protocols

2. Evaluation of key parameters in large field cages such as wild fly sex ratio, degree of lure response of sterile flies, sterile:wild over-flooding ratio and bisexual release should be performed to determine their influence on the effectiveness of simultaneous MAT and SIT

3. Field evaluation of simultaneous MAT and SIT within a pilot or operational setting that includes compatible management practices should be done. Dependent on available resources, recommended treatments in order of priority are:
a. MAT only (control)
b. Simultaneous MAT and SIT (sterile insect release with MAT present) with optimal pre-treatment
c. Sequential MAT and SIT (sterile insect release after removal of MAT) with optimal pre-treatment
d. Simultaneous MAT and SIT (sterile insect release with MAT present) without semiochemical exposure
e. Sequential MAT and SIT (sterile insect release after removal of MAT) without semiochemical exposure

2. Background situation analysis

2.1.Lures: Genetics, physiology and behaviour

The genera *Bactrocera* and *Zeugodacus* (Diptera: Tephritidae: Dacinae: Dacini) include nearly 40 species of economic concern, the most important of which to this CRP are *B. carambolae* (Drew and Hancock), *B. dorsalis* (Hendel), *B. tryoni* (Froggatt), *B. zonata* (Saunders), *Z. cucurbitae* (Coquillett) and *Z. tau* (Walker). Male flies of these species are usually attracted to one of a number of phenylbutanoid or phenylpropanoid compounds (Drew & Hooper, 1981; Fletcher, 1987). For several dacine species there are well documented male mating advantages gained by males after feeding on the lures, mediated (entirely or in part) through a modification of the male pheromone (e.g., Tan & Nishida, 1995). These observations suggest that male attraction to phenylpropanoid or phenylbutanoid compounds is related to sexual activity and can be explained by their similarity to compounds that play a role in the mating systems of these flies. Natural examples of these chemicals (referred to as "semiochemicals" for brevity) include raspberry ketone (RK), eugenol, methyl eugenol (ME), zingerol and zingerone (ZG), which are found across a wide range of plant orders (Raghu, 2004). However, the effect of these semiochemicals on mating is not consistent across these species. Additionally, ZG has been shown to increase metabolism in *B. tryoni*, but not enhance the attractiveness of its pheromone (Kumaran et al., 2014). This is just one example of many which show, or suggest, that the male lures have multiple behavioural and physiological impacts on the fruit flies of concern.

Due to their attractiveness to males of some *Bactrocera* and *Zeugodacus* species, ME and CL are highly effective lures in traps (Raghu, 2004) that are also used for "male annihilation technique" (MAT) (Vargas et al., 2014). MAT is a behavioural control tactic that involves the attraction and killing of target insects. Such is the attractiveness of ME and CL for males of some species, MAT is highly effective for their suppression (e.g., Bateman et al., 1966) and even eradication in some circumstances (e.g., Itô, 2005; Manrakhan et al., 2011). For this reason, MAT can be used prior to sterile insect technique (SIT) programmes to reduce the abundance of wild males (Vargas et al., 2014). Sterile males are then released to replace the wild males in the population, which ensures a high ratio of sterile to wild males in the treatment area and improved population suppression due to limited competition (Klassen, 2005). This sequential application of MAT followed by SIT was believed to be necessary because if MAT and SIT were to be used simultaneously, released sterile males could be attracted to MAT baits to the same extent as wild males.

However, it has been proposed that MAT and SIT may be applied at the same time (Barclay et al., 2014). This simultaneous application may be possible due to the effects of semiochemical exposure on subsequent attractiveness of male dacines to the same or other phenylpropanoids/ phenylbutanoids. Evidence to date suggests that males need to be exposed to one of these chemicals only once in their life to accrue reproductive benefits. Thereafter, the response of males to lures is suppressed. For example, exposing *B. dorsalis* to ME for as little as 30 minutes when sexually mature can lead to reduced capture probability of males by approximately 80% (Shelly, 1994). This effect can last for over 30 days (Shelly, 1994). Interestingly, it may not even be necessary to feed a lure to sterile males before release to reduce their attraction to it. In *B. tryoni*, feeding on RK in an agar block diet containing sucrose and hydrolysed yeast for 48 hours after adult eclosion led to reduced attraction to cue-lure in the laboratory (Akter et al., 2017a), and traps baited with CL in field cages and a commercial orchard (Khan et al., 2017b). Mathematical modelling suggests that the release of sterile males pre-treated with

phenylpropanoids should reduce their attraction to MAT baits, while simultaneously improving their mating success with wild females due to improved sexual performance and lower levels of competition from wild males (Barclay et al., 2014). As such, the simultaneous application of MAT and SIT is proposed to lead to a situation where "male replacement" occurs, with wild males being suppressed by MAT and wild females mating with sterile males that remain in the field. However, further empirical data are required to demonstrate the effectiveness of the simultaneous application of MAT and SIT.

In order to develop operational strategies for simultaneous MAT with SIT, the underlying genetics, behaviour and physiology of the lure effects must be documented. Previous research is clear that these effects are not consistent across species, and must be independently researched for each species of concern. Even for the well-studied *B. dorsalis*, it is now clear that the modified pheromone effect is only one part of a larger story. However, when comparing such studies, it is often difficult to determine how much of the variation across species is due to biological variation, and how much is due to different experimental approaches. Overcoming this problem is an essential role for this CRP.

When considering the integration of MAT and SIT, it is important to recognise that the male lures have two fundamentally different properties, both of which must be assessed. The first is their attractive properties. MAT works by using a male lure to attract an insect to a poison. Thus, the CRP needs to consider the male lures for their olfactory attraction, how this varies between fly species, the lure type, and local environmental conditions. The second aspect of lures to be considered is how they modify the behaviour and physiology of flies after lure exposure (either via feeding or aromatherapy). For example, two critical physiological components which must be assessed is how lure exposure changes physiological development rate and subsequent response rate to later exposure of the same or different lure.

Given this background, our long-term goals are to:

1. Understand the differential sensitivity of different lure types to different fly species and use this information to inform recommendations for the field application of MAT against target species.
2. Determine how exposure of larvae or adults to different lures modifies their subsequent development rate and propensity to response to lures (either the same lure or different lures).
3. Provide behavioural, physiological and ecological insights that will improve modelling of the field phenology of the pests and the integration of MAT and SIT for their sustainable control.

Achieving these outcomes will require substantial advances in our knowledge of the mechanisms of lure response. We will address these long-term goals through research on:

1. Pre-release treatment methodology for reduced lure response
2. Effects of pre-release treatments on fly performance
3. Novel attractants - microbiota, cuticular lipids, and plant extracts
4. Selection for non-responders
5. Mechanisms of lure response
6. Semi-field and field assessments of SIT +/- MAT

2.2. Individual plans

To achieve the long-term goals posed above, participants in this RCP have committed to pursue research on five sub-themes. The six sub-themes will be addressed by using similar approaches on different *Bactrocera* and *Zeugodacus* species (Table 1).

Table 1. Sub-thematic areas being addressed by researchers to assess the feasibility of combined MAT and SIT and the respective insect pest to be studied.

Country	Researcher leader	Species
1. Pre-release treatment methodology for reduced lure response		
Australia	Bishwo Mainali	*Bactrocera tryoni*
Bangladesh	Mahfuza Khan	*Bactrocera dorsalis, Zeugodacus cucurbitae, Zeugodacus tau*, Bactrocera zonata*
China	Bo Wang	*Bactrocera dorsalis*
France	Vincent Jacob	*Bactrocera dorsalis*
India	Ashok Hadapad	*Bactrocera dorsalis, Zeugodacus cucurbitae*
Kenya	Shepard Ndlela	*Bactrocera dorsalis*
Malaysia	Suk Ling Wee	*Bactrocera dorsalis, Zeugodacus cucurbitae, Zeugodacus tau*, Bactrocera carambolae*
Mauritius	Nausheen Patel	*Bactrocera dorsalis, Zeugodacus cucurbitae, Bactrocera zonata*
Pakistan	Ihsan ul Haq	*Bactrocera dorsalis*
South Africa	Christopher Weldon	*Bactrocera dorsalis*
Thailand	Suksom Chinvinijkul	*Bactrocera dorsalis, Bactrocera correcta*
USA	Sheina Sim	*Bactrocera dorsalis*
Vietnam	Hien Nguyen	*Bactrocera correcta*

Country	Researcher leader	Species
2. Effects of pre-release treatments on fly performance		
Australia	Bishwo Mainali	*Bactrocera tryoni*
Australia	Olivia Reynolds	*Bactrocera tryoni*
Bangladesh	Mahfuza Khan	*Bactrocera dorsalis, Zeugodacus cucurbitae, Zeugodacus tau*, Bactrocera zonata*
Brazil	Cristiane Ramos de Jesus	*Bactrocera carambolae*
China	Bo Wang	*Bactrocera dorsalis*
Israel	Yoav Gazit	*Bactrocera zonata*
Malaysia	Suk Ling Wee	*Bactrocera dorsalis, Zeugodacus cucurbitae, Zeugodacus tau*, Bactrocera carambolae*
Mauritius	Nausheen Patel	*Bactrocera dorsalis, Zeugodacus cucurbitae, Bactrocera zonata*
Pakistan	Ihsan ul Haq	*Bactrocera dorsalis*
South Africa	Christopher Weldon	*Bactrocera dorsalis*
Thailand	Suksom Chinvinijkul	*Bactrocera dorsalis, Bactrocera correcta*
Vietnam	Hien Nguyen	*Bactrocera correcta*
USA	Sheina Sim	*Bactrocera dorsalis*
3. Novel attractants - microbiota, cuticular lipids, and plant extracts		
Australia	Anthony Clarke	*Bactrocera dorsalis, Bactrocera tryoni*
Brazil	Cristiane Ramos de Jesus	*Bactrocera carambolae*

Country	Researcher leader	Species
Czechia	Lucie Vaníčková	*Bactrocera & Zeugodacus*
France	Vincent Jacob	*Bactrocera dorsalis*
India	Ashok Hadapad	*Bactrocera dorsalis, Zeugodacus cucurbitae*
Malaysia	Suk Ling Wee	*Bactrocera dorsalis, Bactrocera carambolae*
USA	Sheina Sim	*Bactrocera dorsalis*
4. Selection for non-responders		
Australia	Bishwo Mainali	*Bactrocera tryoni*
Israel	Yoav Gazit	*Bactrocera zonata*
Malaysia	Alvin Hee	*Bactrocera dorsalis*
5. Mechanisms of lure response		
Australia	Anthony Clarke	*Bactrocera dorsalis, Bactrocera tryoni, Bactrocera cacuminata*
Australia	Bishwo Mainali	*Bactrocera tryoni*
Australia	Olivia Reynolds	*Bactrocera tryoni*
Malaysia	Alvin Hee	*Bactrocera dorsalis*
USA	Sheina Sim	*Bactrocera dorsalis*
6. Semi-field and field assessments of SIT +/- MAT		
Australia	Olivia Reynolds	*Bactrocera tryoni*

Country	Researcher leader	Species
Australia	Bishwo Mainali	*Bactrocera tryoni*
Bangladesh	Mahfuza Khan	*Bactrocera dorsalis*
China	Bo Wang	*Bactrocera dorsalis*
France	Helene Delatte, Laura Moquet	*Bactrocera dorsalis*
India	Ashok Hadapad	*Bactrocera dorsalis*
Israel	Yoav Gazit	*Bactrocera zonata*
Mauritius	Nausheen Patel	*Bactrocera dorsalis, Bactrocera zonata, Zeugodacus cucurbitae*
New Zealand	Lloyd Stringer	*Bactrocera tryoni, Bactrocera dorsalis, Bactrocera zonata*
Pakistan	Ihsan ul Haq	*Bactrocera dorsalis*
South Africa	Christopher Weldon	*Bactrocera dorsalis*
Thailand	Suksom Chinvinijkul	*Bactrocera dorsalis, Bactrocera correcta*
USA	Sheina Sim	*Bactrocera dorsalis*
Vietnam	Hien Nguyen	*Bactrocera correcta*

*****_Z. tau_ species complex

2.3.Sub-themes

2.3.1. Pre-release-treatment methodology for reduced lure response

The male annihilation technique (MAT) can be a very effective component of integrated pest management (IPM) programs (Benelli et al., 2014; Steiner et al., 1965; Vargas et al., 2015) and has been demonstrated to be effective for the population suppression and eradication of tephritid fruit flies (Koyama et al., 1984; Steiner et al., 1970; Vargas et al., 2000). This technique relies on

a powerful male lure to attract wild and fertile males to MAT devices where they are removed from the reproductive population via insecticide (Vargas et al., 2003). Unfortunately, the release of sterile males for the sterile insect technique (SIT) represents an incompatibility between these two components of an IPM program. To maintain realistic and effective overflooding ratios between wild and sterile males, the lure response of mass-released sterile males must be reduced.

Previous studies have shown phenotypic plasticity in male lure response in *Bactrocera dorsalis*. Adult flies exposed to methyl eugenol for as little as 30 seconds demonstrate a lowered response to methyl eugenol in mark-release-recapture trials and they demonstrate a lowered response several days after exposure (Shelly, 1994). Analogously, Manoukis et al. (2018) showed that adult *B. dorsalis* emerging from host fruit in the genus *Terminalia* (common name: tropical almond) are not responsive to methyl eugenol. This indicates a different mechanism for inducing a reduced lure response and that the flies emerging from *Terminalia* were exposed to a response reducing agent at the larval stage. Studies outside of Tephritidae have also shown the potential for aversion therapy to condition flies to demonstrate a reduced response to olfactory stimuli in *Drosophila melanogaster* (Malik & Hodge, 2014). These previous observations suggest that a variety of strategies may be effective in inducing a reduced lure response in tephritid fruit flies. Thus, further study is required so that the most effective methodology can be implemented to synergistically combine MAT with SIT.

Gaps:

1. Effective methodology for pre-treating flies to reduce lure response unknown.
2. Physiological response of flies to treatments for reduced lure response unknown.

2.3.2. Effects of pre-release-treatment on fly performance

Male flies from the tribe Dacini are usually attracted to certain chemical compounds referred to as phenyl-propanoids or -butanoids (Drew & Hooper, 1981; Fletcher, 1987). Feeding on these chemicals improves male sexual performance by increasing the rate of sexual maturation, attractiveness of male pheromone, calling rate, and/or mating propensity (e.g., Akter et al., 2017b; Raghu & Clarke, 2003; Shelly & Nishida, 2004; Wee et al., 2007). This leads to improved mating competitiveness relative to males without access to the chemical (Haq et al., 2014; Shelly et al., 2010). These observations suggest that male attraction to phenylpropanoid compounds is related to sexual activity and can be explained by their similarity to compounds that play a role in the mating systems of these flies (Tan & Nishida, 1995, 2000, 2007). Natural examples include raspberry ketone, eugenol, methyl eugenol, zingerol and zingerone, which are found across a wide range of plant orders (Raghu, 2004).

Some studies that have focused on the effects of phenyl-propanoid or -butanoid exposure on the mating behaviour of male dacines show higher levels of activity in treated males. For example, methyl eugenol-fed male *B. dorsalis* (reported as *B. papayae* Drew & Hancock) exhibited earlier calling behaviour than their unfed counterparts, which increased attraction of females (Hee & Tan, 1998). This was also accompanied by an increase in locomotor behaviour (Hee & Tan, 1998). In *B. cacuminata* (Hering), methyl eugenol-fed males were not preferred by females in two-choice tests, but still secured more matings than those not fed methyl eugenol (Raghu & Clarke, 2003). These observations suggested a potential physiological fitness benefit of phenyl-propanoid or -butanoid exposure rather than an effect on the male pheromone.

Gaps:

1. The effects of feeding or exposure to phenyl-propanoids or -butanoids in a selected range of dacine species, with a focus on behaviour, reproductive success, and survival unknown.

2.3.3. Novel attractants - microbiota, cuticular lipids, and plant extracts

Male dacine flies are strongly attracted to methyl eugenol (ME), anisyl acetone, cue-lure, raspberry ketone (RK) and zingerone etc (Tan & Nishida, 2012). Lures based on these compounds are being used in pest management programmes in MAT devices. However, there are about 200 species of fruit flies, which do not respond to these potent lures (Tan & Nishida, 1996). In addition, the cost of the chemicals, the banned use of ME in the European Union, and chemical contaminants of the lure may limit the use of these attractants. Thus further search for novel compounds as attractants is needed. Exploring novel attractants has great potential for efficient pest management. Certain compounds are known to be available in several plant species. *Bactrocera* fruit flies are attracted to several orchid flowers, host fruits and other plant parts (Tan & Nishida, 1996). A series of phenylpropanoids and phenylbutanoids have been identified from a number of *Bulbophyllum* species. A sesquiterpene hydrocarbon and *β*-caryophyllene have been identified from the rectal glands of wild *B. correcta* males (Zhang et al., 2019). Some of the unknown volatiles in host plants parts and gut microbiota may be strongly associated with dacine species. It has been observed that fruit fly host plants and byproducts of certain gut microbiota or plant extracts attract the adults of various *Bactrocera* species (Hadapad et al., 2016; Wang et al., 2014). Thus, it is essential to study the novel chemicals present in plant extracts, microorganisms associated with insects and their body parts. In Tephritidae, cuticular lipids have been previously used for species delimitation and chemotaxonomy of cryptic species complexes, e.g. *Anastrepha fraterculus* complex, *Ceratitis* FAR complex and *B. dorsalis* complex (Vaníčková et al., 2015a; Vaníčková et al., 2015b; Vaníčková et al., 2017; Vaníčková et al., 2014). Detailed chemical analyses of cuticular composition presents an important method for estimation of fitness and age of the fruit fly populations used in eradication programs. These studies are still missing for the genus *Zeugodacus* and for some of the economically important species of the *Bactrocera* genus only limited information are available.

Gaps:

1. Studies on sex- and age-dependent production of cuticular lipids, gut microbiota, and response to host plant extracts are essential for understanding of the role of these compounds in the chemical communication in dacine fruit flies.

2.3.4. Selection for non-responders

In any area-wide MAT-SIT programme against tephritid pests, the availability of a potent male attractant and mass-reared sterile male flies that are of ecological competence is necessary to ensure operational viability. Thus, in the case of the oriental fruit fly, *B. dorsalis*, for decades the use of insecticide bait traps containing the potent male lure, methyl eugenol in MAT followed by successful releases of mass-reared sterile males to compete with wild males for feral females has resulted in reduction in the population of wild flies (Steiner et al., 1970). However, whilst MAT and SIT are independent control tactics, current practice of combining both techniques as a MAT-SIT sequence results in sterile males themselves getting killed following their attraction to feed on methyl eugenol contained in those insecticide-laced MAT devices. Thus, overcoming this problem will incur a significant amount of cost and time in mass-rearing very high numbers

of sterile males and removal of all those MAT devices in the field prior to releases of those sterile males.

In circumventing the issues faced in a sequential MAT-SIT programme, simultaneous MAT-SIT application has been suggested instead as a more cost-effective tactic (Barclay et al., 2014). Nevertheless, the problem of sterile males being attracted to MAT devices continues to persist. Though habituation can be induced in male flies based on lure feeding (Shelly, 1994), incorporation of lure in the adult diet (Akter et al., 2017a; Khan et al., 2017a) or host fruit extracts in fly larvae diet (Manoukis et al., 2018), permanent diminished attraction to lure as an inheritable phenotypic trait or other means of treatment will be ideal. Interestingly, based on the work of Itô and Iwahashi (1974) and Shelly (1997) that lines of non-ME-attracted male *B. dorsalis* can be developed, the selection of non-lure-attracted males ("non-responders") as candidates for sterility treatment and mass-releases in SIT is possible. This warrants their evaluation for ecological competence against lure-attracted mass-reared and feral males respectively.

Gaps:

1. Lines of non-responders have not been evaluated for fitness as candidates for mass-rearing and releases as sterile males.

2.3.5. Mechanisms of lure response

The genera *Bactrocera* and *Zeugodacus* include nearly 40 species of economic concern, the most important of which to this CRP are *B. dorsalis, B. carambolae, B. tryoni, B. zonata, Z. cucurbitae* and *Z. tau*. The effect of the male lures (i.e. methyl eugenol, cue-lure, raspberry ketone, zingerone) are not consistent across these species. For several species there are well documented male mating advantages gained by males after feeding on the lures, mediated (entirely or in part) through a modification of the male pheromone. However, zingerone feeding in *B. tryoni* has been shown to increase male mating success not through an enhanced male pheromone, but rather through an increase in metabolic activity and general fly activity, This is just one example, of many now in the literature, which infer that the male lures have multiple behavioural and physiological impacts on dacines.

This CRP is investigating increased growth rate and more rapid sexual maturity in flies following lure feeding, as well as utilising the observed fact that fly response to a lure (exposed in MAT devices) decreases following lure exposure as either a larva or adult fly. However, only for *B. tryoni* has a metabolic effect of lure feeding been demonstrated: it was not found when looked for in *Z. cucurbitae*. Knowing the mechanistic basis of how lures impact on the flies will provide a firmer basis for developing efficacious MAT/SIT combinations and exposure treatments, as well as providing insights into what might be expected if studies are begun on pest species for which no prior behavioural information exists (e.g. *B. minax, Z. cucumis*). Such fundamental research may also lead to entirely new fields of application: as for example has the recognition that zingerone is a metabolism enhancer in some fruit flies.

Gaps:

1. There is no mechanistic explanation as to why fly response to a lure is decreased after prior lure feeding.
2. The underlying genetic and physiological mechanisms which lead to phenotypic changes have not been studied.

2.3.6. Semi-field and field assessments of SIT +/- MAT

Certain dacine males show strong responses to different phytochemicals or their synthetic analogs such as methyl eugenol (ME), raspberry ketone (RK)/cue-lure and zingerone (Shelly et al., 2008; Tan & Nishida, 2012; Weldon et al., 2008). Feeding on these chemicals improves male sexual performance by increasing the rate of sexual maturation, the attractiveness of male pheromone, and mating propensity leading to improved mating competitiveness relative to males without access to the chemicals (Akter et al., 2017b; Khan et al., 2017b; Kumaran et al., 2013; Perez-Staples et al., 2007; Raghu & Clarke, 2003; Shelly & Nishida, 2004). These compounds are highly attractive to the males of tephritid fruit flies and are thus used in trapping programmes to identify incipient infestations or in MAT to suppress or eradicate populations (Manoukis et al., 2019). MAT has been used as a management tool against many dacine species, however, if combined with other control methods such as sterile insect releases and/or protein bait sprays, it will help the suppression and/or eradication of wild populations.

Persistence of MAT devices in the environment often hinders the strategic implementation of MAT and SIT in sequence (Akter et al., 2017a). The residues of the MAT device may attract and kill the released sterile males thereby weakening the control levels by SIT. Substantial advantages in control levels can be achieved if MAT and SIT could be used simultaneously through the release of male flies with the suppressed response to the lures used in MAT (Barclay et al., 2014). Several studies reported that the male tephritid either exposed or fed the phytochemicals showed a reduced response to the lures used in MAT, such as *B. dorsalis* males to methyl eugenol (Shelly, 1994) and to RK and its synthetic analogue cue-lure for fertile (Akter et al., 2017a) and sterile (Khan et al., 2017a) *B. tryoni* males. The evidence that flies pre-treated with male lures can have a lasting effect of reduced responsiveness to those lures, provides a foundation for the development of the simultaneous deployment of MAT and SIT. In this scenario, the released sterile males replace the wild males in the population, which have also been reduced through MAT; this ensures a high ratio of sterile to wild males in the treatment area and improves population suppression. However, studies on this potentially synergistic relationship between the simultaneous application of MAT and SIT to improve the efficacy of fruit fly management is sparse, warranting assessment in semi-field and field conditions.

Gaps:

1. Logistical problems involved in the scaling up of production of pre-treated flies for simultaneous use of MAT and SIT has not been addressed.
2. There is currently no empirical evidence on the relative improvement of the simultaneous application of MAT and SIT with non-lure-responsive flies for population suppression.

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APPENDIX 1. Individual 5-year working plans

APPENDIX 2. List of participants

First RCM on "Assessment of Simultaneous Application of SIT and MAT to Enhance *Bactrocera* Fruit Fly Managements"
15-19 July 2019, Vienna, Austria

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APPENDIX 3. Agenda

FIRST FAO/IAEA RESEARCH COORDINATION MEETING ON:
Assessment of Simultaneous Application of SIT and MAT to Enhance *Bactrocera* Fruit Fly Management
15–19 July 2019, Vienna, Austria
Vienna International Centre, (Room M4 – M Building)

AGENDA

MONDAY, 15 JULY 2019
08:00 – 08:30 **Identification and registration at VIC Gate (next to subway station U1)**
08:30 – 08:45 **Rui Cardoso Pereira/Carlos Cáceres:** Welcome statement and goals of the meeting
08:45 – 09:00 **Carlos Cáceres:** Logistics and administrative issues

SESSION I: Lures: Genetics, physiology and behaviour (Chairperson: Stringer)
09:00 – 09:30 **Anthony Clarke**: Fruit fly mating systems and male lures
09:30 – 10:00 **Yoav Gazit**: The effect of various physiological parameters on the respond of *Bactrocera zonata* males to methyl eugenol
10:00 – 10:30 **Alvin Hee**: Developing non-methyl eugenol-responding male oriental fruit flies supplemented with semiochemical exposure for improved sterile insect technique

COFFEE BREAK

11:30 – 12:00 **Hien Nguyen:** Assessment the impact of adult diet and methyl eugenol on male sexual success of *Bactrocera correcta*

LUNCH

13:00 – 13:30 **Nausheen Patel:** Effect of semiochemicals on calling behaviour of *Bactrocera zonata* and *Zeugodacus cucurbitae* and mating competitiveness of laboratory reared Bactrocera *zonata*
13:30 – 14:00 **Cristiane Ramos de Jesus:** Effect of semiochemical on carambola fruit fly behaviour, survival, and lure response to improve pest management in Brazil
14:00 – 14:30 **Olivia Reynolds**: Semiochemical supplementation accelerates sexual maturation and improves mating performance of sterile male Queensland fruit fly, *Bactrocera tryoni* (Froggatt)
14:30 – 15:00 **Sheina Sim:** Screening methyl eugenol-related chemicals for induction of reduced lure response in male *Bactrocera dorsalis*: from fruit extracts to field application

COFFEE BREAK

15:30 – 16:00 **Lucie Vaníčková:** Cuticular hydrocarbons: towards new chemotaxonomic and chemo-ecological tools to manage *Zeugodacus* and *Bactrocera* pests?
16:00 – 16:30 **Suk Ling Wee:** Improvement of *Bactrocera carambolae* management by insect growth regulator and semiochemicals for simultaneous application of SIT and MAT
16:30 – 17:00 **Vincent Jacob:** Cross-species comparison of fruit fly olfactory perception of host fruits as a way of exploring new attractive blends

TUESDAY, 16 JULY 2019
SESSION II SIT and MAT integration: laboratory and field assessments (Chairperson: Suk Ling Wee)
08:30 – 09:00 **Ashok Hadapad:** Evaluation of integrated male annihilation techniques (MAT) and sterile insect technique (SIT) to enhance the performance of sterile males of *Bactrocera* species
09:00 – 09:30 **Ihsan ul Haq:** Towards combined use of MAT and SIT for management of *Bactrocera* (Diptera: Tephritidae) fruit flies: Developing methods of delivering methyl eugenol, hormones, and diet supplements
09:30 – 10:00 **Mahfuza Khan:** Assessment of simultaneous application of SIT and MAT to enhance management of *Zeugodacus cucurbitae* (coq.) and *Bactrocera dorsalis* (Hendel) in Bangladesh

COFFEE BREAK

10:30 – 11:00 **Shepard Ndlela**: Semiochemicals for pre-release treatment of *Bactrocera dorsalis* and relative response to male lures in semi field conditions
11:00 – 11:30 **Lloyd Stringer:** Estimating the effect of the simultaneous use of male annihilation technique and sterile *Bactrocera* release on population reduction rates
11:30 – 12:00 **Suksom Chinvinijku:** Cost-effective treatments support the Male Annihilaton Technique and the Sterile Insect Technique for the Establishment of a Fruit Fly Low Prevalence Area in Thailand

LUNCH

13:00 – 13:30 **Phil Taylor:** Pre-release Raspberry Ketone (RK) feeding and Non-responder Selection as approaches for developing cuelure non-responsive Queensland fruit flies for combined SIT and MAT
13:30 – 14:00 **Bo Wang:** Effect of Methyl Eugenol feeding on re-feeding and mating behaviour
14:00 – 14:30 **Christopher Weldon:** Simultaneous application of MAT and SIT for management of *Bactrocera dorsalis* in South Africa: environmental and physiological considerations
14:30 – 15:00 **Laura Moquet:** Invasion of *Bactrocera dorsalis* in Reunion Island

COFFEE BREAK

15:30 – 16:00 **Helene Delatte:** Evaluation of the feasibility of the management of the invasive fruit fly pest *Bactrocera dorsalis* on mango in Réunion using the sterile insect technique
16:00 – 17:00 General discussion and formation of working groups

WEDNESDAY, 17 JULY 2019
SESSION III: Review of the individual proposals (Chairperson: Carlos Cáceres and Group Leaders)
08:30 – 10:00 Working Groups: Preparing individual plans for the 5 year of the CRP and for the next 18 months

COFFEE BREAK

10:30 – 12:00 Working Groups: Preparing individual plans

LUNCH

13:00 – 15:00 Working Groups: Preparing individual plans

COFFEE BREAK

15:30 – 17:00 Working Groups: Preparing individual plans

THURSDAY, 18 JULY 2019
SESSION IV: RCM report (Chairperson: Carlos Cáceres and Group Leaders)
08:30 – 10:00 Review and adjustment of the logical framework

COFFEE BREAK

10:30 – 12:00 Review and agreement on content of RCM report (main achievements and recommendations)

LUNCH

13:00 – 16:30 Visit to the Insect Pest Control Laboratory, Seibersdorf

18:30 **GROUP DINNER (Zur Alten Kaisermühle)**

FRIDAY, 19 JULY 2019
SESSION IV cont.: RCM report (Chairperson: Carlos Cáceres and Group Leaders)
08:30 – 10:00 Review and agreement on content of RCM report (main achievements and recommendations)

COFFEE BREAK

10:30 – 12:00 Review and agreement on content of RCM report (main achievements and recommendations).

LUNCH

13:00 – 15:00 Presentation of the final RCM report (main achievements and recommendations)

APPENDIX 4. Abstracts of presentations

Fruit fly mating systems and male lures

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Seminal research from Malaysia and Hawaii in the mid-1990s identified that male *B. dorsalis* and *Z. cucurbitae* gained a mating advantage following lure feeding through modification of the male pheromone. However, as early as 2002 we identified that the modified pheromone model did not hold for the Australian ME responsive fruit fly *B. cacuminata*. For this species a mating advantage was detected only weeks after initial feeding, but we did find direct evidence that the species used ME as a mate rendezvous site at dusk. Since then a small but growing body of evidence shows that while the lures are invariably associated with the mating systems of *Bactrocera* species, altered pheromones are only one part of the story. This talk will illustrate how, while pursuing research on the *B. tryoni* mating system, we identified that for this species the lures have a metabolic enhancement effect. As for other lure research, this effect is not automatically applicable to other species, and needs to be independently researched for each species of interest. However, even for the well-studied *B. dorsalis*, a possible metabolic impact of ME may explain published observations by others in the CRP which are not consistent with the well proven pheromone effect: for example lure-fed *B. dorsalis* males calling earlier than control males; an ME effect transferred from larvae to adults; and a mating advantage gained by males exposed to ME only as a volatile.

The effect of various physiological parameters on the response of *Bactrocera zonata* males to methyl eugenol

Yoav Gazit[1], David Nestel[2]:

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Poor information exists on the attraction of the male of the peach fruit fly *Bactrocera zonata* (PFF) to methyl eugenol (ME). This knowledge is important for the development of control strategies that combine the sterile insect technique (SIT) and the Male Annihilation Technique (MAT). We propose to characterize adult male PFF response to ME as affected by: (a) age, and sexual maturation; (b) the adult diet; (c) pre-exposure to ME; and (d) the sterility status, and irradiation dose. Males PFF of different ages, exposed or not to ME, and fed with different adult diets, will be obtained from a PFF colony maintained under quarantine conditions in the Plant protection and Inspections Services (PPIS) facilities in Israel. The attraction to ME traps will be inquired by release-recapture experiments conducted under field conditions inside four double-screen cages (12 X 10 X 4 m) covering mature citrus-trees (12 trees/ cage) simulating natural mini-orchards. Physiological state (e.g., nutritional state and ME pre-exposure) will be induced, and its effects upon metabolism and development evaluated by food ingestion, metabolite (e.g., lipid and protein) contents and gonadal development. Sterility will be induced using gamma

radiation by sub-sterilizing doses (20, 40 and 60 Gy) and sterilizing doses (100 Gy) The last part of the project will evaluate how sterile flies' attractiveness to ME is affected by ME-pre-exposure before release and by diet type.
Our capacity:
Equipped laboratories for experimentation in fruit fly behaviour and physiology exists in both participating institutions. A PFF colony produces ~10,000 pupae/ week. Four double-screen field cages bearing mature citrus trees.
We expect that this investigation will provide insights on the interaction of male physiological status and attractiveness to ME in the peach fruit fly.

Developing non-methyl eugenol-responding male oriental fruit flies supplemented with semiochemical exposure for improved sterile insect technique

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The Oriental fruit fly (OFF), *Bactrocera dorsalis* (Hendel) is one of the most destructive insect pests of fruits and vegetables worldwide due to its wide host range coupled with its highly invasive nature. Hitherto, whilst the OFF continues to pose an imminent threat to OFF-free countries such as Australia and continental United States, this species has already been established in sub-Saharan Africa and currently invading central China, the top fruit producing country in the world. Current control of OFF is still heavily reliant on the male annihilation technique (MAT), in which a highly attractive male lure, methyl eugenol (ME) supplemented with insecticides are utilized in bait traps. With the development of sterile insect technique (SIT) in which mass-reared sterile male flies are released into the field to outcompete feral males in mating resulting in zero progeny produced, sequential use of MAT and SIT has resulted in some successes in area-wide control programmes. However, the effectiveness of SIT is undermined when sterile males are also attracted to those ME-baited traps and killed. Thus, considering wide interest to evaluate the potential synergistic relationship for simultaneous MAT-SIT application, we propose the idea to use non-ME-attracted OFF males (non-responders) to prevent or reduce attraction to ME-baited traps but can still be treated with certain semiochemicals such as zingerone (ZN) to increase male mating competitiveness. Our previous work has shown that ZN-treated OFF males demonstrated higher mating competitiveness compared with non-ZN-fed males. In this proof-of-concept 5-year project, a stable line of fit non-responders will be obtained from the wild (1st year of CRP), and tested for mating fitness compared with responders from same cohorts as well as laboratory strain (2nd -3rd year of CRP). The attraction of those non-responders to semiochemicals such as ZN will be evaluated (3rd -4th year of CRP) and tested in mating competition against non-ZN-fed males from same cohorts as well as laboratory strain. Further changes in the transcriptome and proteome profiles of those non-responders especially in relation to ZN exposure is expected to reveal further insights on those receptor expression mechanisms (4th -5th year of CRP). This will certainly facilitate identification of specific

odourant receptors, building on our current work on the identification of genes and proteins in the antennae and maxillary palps of ME-exposed OFF, that can be manipulated for simultaneous MAT-SIT application. Current progress and results obtained for the first year will also be presented.

Assessment the impact of adult diet and methyl eugenol on male sexual success of *Bactrocera correcta*

Trang, V.T.T, Hien, N.T.T.

Plant Protection Research Institute, Viet Nam

Six diet treatments including sugar (T1), protein (T2), a mix protein and sugar (T3), sugar extra Methyl eugenol (T4); protein extra Methyl eugenol (T5) and a mix of protein and sugar extra Methyl eugenol (T6) were conducted to rear *B. correcta*. The highest dead rate of adult was found at 4 days old fed with T2 and T5 as the proportion is 100%. Similarly, at 9 days old it was reported to die most if using T1 (20,33%). For mating research, apply above treatments until the male reached 10 days old, result showed that the percentage had varied from 1,67% to 18,33%.

Effect of Semio-chemicals on calling behaviour of *Bactrocera zonata* and *Zeugodacus cucurbitae* and mating competitiveness of laboratory reared peach fruit fly *Bactrocera zonata*

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Fruit flies (Diptera: Tephritidae) are the most destructive and important pests of fruits and vegetables worldwide. Fruit fly problems in Mauritius date back to the beginning of the nineteenth century. The peach fruit fly *Bactrocera zonata* (Saunders) are key pest of mango *Mangifera indica L.*, guava *Psidium guajava* L. and peach *Prunus persica* in Mauritius, while the melon fly *Zeugodacus cucurbitae* (Coquillett) infest cucurbits. The Sterile Insect Technique (SIT) is an important component of many area-wide integrated pest management programs against Tephritid fruit flies and its overall success is highly dependent on the field performance of the mass-reared, sterilized, and released insects. Wing fanning is a predominant male sexual behaviour reported in several *Bactrocera* spp. Male melon flies produce a buzzing sound by vibrating their wings together with release of pheromones that are highly attractive to females. Males of a majority of *Bactrocera* spp.and Zeugodacus spp. are attracted to natural compounds known as semio-chemicals. The objective of this study was to determine the effect of exposure to methyl eugenol and cuelure on (i) calling behaviour of *B. zonata* and *Z. cucurbitae* respectively and on (ii) mating competitiveness of *B. zonata*. Pupae of the respective flies were irradiated at 70 Gys 2 days prior to emergence. Flies were sexed at emergence and kept in a 30 x 30 x 30 cm Perspex fruit fly cage and provided with adult fruit fly diet and water *ad libitum*. On day 2, the male flies were exposed to methyl eugenol (0.5 ml/100 flies). The flies were allowed to feed on

the ME for one hour (08:30 – 09:30). Untreated males from the same cohort of flies were used as control. Wing fanning of the flies was observed between 16:30 to 20:00. A battery operated torch with a red mousseline paper cover was used for observation on nightfall. Four groups of 5 males were observed in gauze cages for calling behaviour on each day until the flies were 32 days old. For the second experiment sterilised *Bactrocera zonata* flies reared on artificial larval gel diet was used. Males were separated after 3 days of emergence (9 days prior to reaching full sexual maturity) and transferred to a Perspex fruit fly cage (30 cm x 30 cm x 30 cm). Marking of males using water-based paint was done one day prior to exposure to methyl eugenol by using a nylon netting to immobilise flies. The male flies were allowed to feed on the methyl eugenol on Day 4 for one hour from 08:30 to 09:30. Semi-wild *B. zonata* flies recovered from incubation of fruits and built up over two generations were used for mating competitiveness test in field cages. 50 ME-fed male flies and 50 untreated male flies were released in the field cage at 16:30 to establish their territories. Thirty minutes later 50 virgin females were released in the field cage. Both the male and female flies were 14 days old. There were 3 replicates. Mating couples from each cage were collected in vials and properly labelled. The time of capture was noted every ten minutes as from 17:00 to 19:30. Wing fanning was more frequent in treated *B. zonata* flies compared to non-treated *B.zonata* flies. Both the time factor (*F= 6.90, df = 21, P= 0.000)* and the treatment factor (exposure to Me) ((*F= 7.41, df = 1, P= 0.013)* were significant. However, the means of the two treatments were not significantly different (T = 1.37, df = 42, P = 0.089) as regards to time of calling. Me-fed male flies started calling earlier on Day 6 as compared to untreated flies which started calling on Day 8. Both the age factor (*F= 5.91, df = 29, P= 0.000)* and the treatment factor was significantly different *(F= 52.98, df = 1, P= 0.000).* Significantly more calling was observed with Me-fed flies (T = 3.92, df = 58, P = 0.000) compared to non-treated flies as regards to age of calling. In the field cage experiment more than 50 % matings were obtained with Me-fed males as compared to 16 % with untreated males (F= *23.31, df = 5, P =0.008).* Me-fed flies started mating earlier (17:10*)* as compared to untreated flies which started mating twenty minutes later. As regards to calling behaviour in *Z. cucurbitae*, more wing fanning was recorded in treated flies compared to non-treated flies. Both the time factor (*F= 68.18, df = 21, P= 0.000)* and the treatment factor (exposure to Cue) ((*F= 14.14, df = 1, P= 0.001)* were significant. However, the means of the two treatments were not significantly different (T = 0.64, df = 42, P = 0.263) as regards to time of calling. Me-fed male flies started calling earlier on Day 7 as compared to untreated flies which started calling on Day 8. Both the age factor (*F= 15.60, df = 29, P= 0.000)* and the treatment factor was significantly different *(F= 27.26, df = 1, P= 0.000).* Significantly more calling was observed with Me Fed flies (T = 1.81, df = 58, P = 0.038) compared to non-treated flies as regards to age of calling. Earlier sexual maturity and enhanced mating competitiveness of ME-fed flies will definitely contribute in improving the effectiveness of a SIT programme for the suppression of the peach fruit fly.

Effect of semiochemical on carambola fruit fly behaviour, survival, and lure response to improve pest management in Brazil

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The introduction of *Bactrocera carambolae* in South America came from the source populations in West Sumatra and Java it was confirmed through microsatellite DNA markers. Suriname and Guiana population is formed by asian immigration. The first record of carambola fruit fly in Suriname was in 1986, French Guiana was in 1989, Guiana was in 1993 and Brazil in was in 1996. The Brazil/French Guiana border is vulnerable with the addition of intense traffic at the border with boat containing people carrying fruits and the road traffic with the recent bridge opening. The control is done by the Ministry of Agriculture, Levestock and Food Supply through Carambola Fruit Fly Eradication Programm using the MAT – Male annihilation technique, destruction of the fruit hosts and also hosts pulverization using spinosad in urban areas. The monitoring is done all over the country with Jackson and McPhail traps. In the Brazilian north region carambola fruit fly is present. Great efforts so that the carambola fruit fly does not disperse to exportation areas. Our proposal aims to fill the gap in knowledge related to the integration of SIT and MAT considering the real advantage of ME and alternative semiochemicals for pre-release treatments on the male lure response, survival after treatment, sexual behaviour, lekking performance, and copulation parameters of *B. carambolae*. Overall programm of work: Daily characterization of *Bactrocera carambolae* behaviour in the laboratory. Effect of clove, copaiba, and andiroba oils on the attraction of *B. carambolae* males. The role of volatile compounds from fruit hosts on the sexual performance of *B. carambolae* males. Effect of volatile compounds on sexual competitiveness and lek performance of *B. carambolae* males under semi-natural conditions. Programm of work for the coming year: Maintenance of the *B. carambolae* colony to be used in the experiments in the Laboratory of Entomology at Embrapa Amapá. Volatile collection from the main fruit hosts of *B. carambolae*. Initial experiments using video recording to evaluate the general behaviour of *B. carambolae* under laboratory conditions. Male lure response of *B. carambolae* to clove, copaiba, and andiroba oils. The role of volatile compounds from fruit hosts on the sexual performance of *B. carambolae* males under laboratory conditions. Preliminary results from sexual competitiveness tests comparing treated and untreated males of *B. carambolae*. Expected outputs: Daily behaviour pattern of *B. carambolae* in laboratory conditions. Sexual behaviour of *B. carambolae* in response to volatiles. Determination of methyl eugenol content in copaiba (*Copaifera langsdorffii*) and andiroba (*Carapa guianensis*) oils. Effect of methyl eugenol extracted from copaiba and Andiroba oils on the attraction of *B. carambolae* males. Effect of host volatiles on the sexual performance of *B. carambolae* males fed with methyl eugenol as larvae. Effect of the host volatiles on the sexual performance of *B. carambolae* males treated with aromatherapy in comparison to methyl eugenol treatment. Contribution with basic information to improve MAT for *B. carambolae* in Brazil. Contribution with basic information to support the future implementation of a SIT programm against *B. carambolae* in Brazil.

Semiochemical supllementation accelerates sexual maturation and improves mating performance of sterile male queensland fruit fly, B*actrocera tryoni* (Froggatt)

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The native Queensland fruit fly, *Bactrocera tryoni* Froggatt (Diptera: Tephritidae) is Australia's most destructive fruit fly species, impacting most commercially produced fruit and fruiting vegetables, impacting our AU$9billion horticultural industry. The sterile insect technique (SIT) is one of the tools used to manage this pest species, however sterility impacts fly performance. The provision of the semiochemical raspberry ketone, known to be attractive to *B. tryoni*, to immature sterile flies pre-release has been shown to both increase their survival, and reduce their response to cue-lure in field studies. This study shows that pre-release dietary supplementation of RK fed to immature sterile males in the days following eclosion can also benefit post-release *B. tryoni* sterile males by accelerating the sexual maturation rate (6 as opposed to 7days) and increasing overall repeated mating performance (decreasing the mating latency period as flies matured and increasing the number of matings with fertile females as flies aged). While RK increased mating competitiveness against wild flies, this did not differ to that of non-RK supplemented sterile flies. Improvements in the effectiveness of sterile flies in the field, could ultimately reduce the number of flies required in SIT programs.

Screening methyl eugenol-related chemicals for induction of reduced lure response in male *Bactrocera dorsalis*: from fruit extracts to field application

Nicholas Manoukis, Dong Cha, Sheina Sim

USDA-ARS Daniel K. Inouye US Pacific Basin Agricultural Research Center

Combining the strengths of the male annihilation technique (MAT) with the virtues of sterile insect technique (SIT) holds promise for efficient and cost-effective control and eradication of *Bactrocera* fruit flies and possibly other species for which there exist powerful male attractants. A synergy of the two techniques would increase the relative reproductive fitness of released sterile males by rendering them less- or non- responsive to the powerful male lure-baited traps that attract and kill their wild competitors. A previous study suggests that *Bactrocera dorsalis* larvae reared in host fruit in the genus *Terminalia* (common name: tropical almond) result in adult males that are less responsive to the powerful male lure methyl eugenol (ME). This provides evidence for a potential mechanism for this strategy but requires further study to enable implementation as part of an integrated pest management (IPM) program. We are in the process of building upon this research by identifying the compounds in *Terminalia* fruit that may result in reduced lure response through the integration of chemical ecology, behavioral ecology, and genetics. Currently, we are conducting experiments to optimize the administration of ME and ME analogue treatments to the *B. dorsalis* Genetic Sexing Strain DTWP. In addition, experiments testing the effect of larval diet supplemented with ME are underway. We anticipate this research to result in a novel additive that can be introduced to larval diet to render mass-reared sterile male *B. dorsalis* temporarily resistant to methyl eugenol attractants.

Cuticular hydrocarbons: towards new chemotaxonomic and chemo-ecological tools to manage *Zeugodacus* and *Bactrocera* pests?

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Genus *Bactrocera* Macquart (Diptera: Tephritidae) belongs to the family of true fruit flies and contains over 500 species occurring in South-east Asian and Pacific regions. The genus *Zeugodacus* currently includes 192 species. Most species within this genus are restricted to the Oriental and Australasian Regions, with a few species reaching into the eastern Palearctic in China and Japan, except for *Z. cucurbitae* which was introduced into other parts of the world. *Bactrocera dorsalis, B. carambolae, B. oleae* and *Zeugodacus cucurbitae* are considered one the most destructive pests of agricultural crops worldwide. Despite their economic importance, little is known about the production of cuticular hydrocarbons in both sexes of *Bactorcera* spp. and *Zeugodacus*. Investigating the chemical ecology of these important pests may allow shedding light on mate choice mechanisms, adding useful information to improve behavior-based control strategies. In this research we propose to investigate in detail the epicuticular composition of *Bactrocera* spp. and *Z. cucurbitae* males and females using two-dimensional gas chromatography coupled to mass spectrometric detection and multivariance factorial analysis together with behavioral assays. This study will add basic knowledge to the chemical ecology of the mentioned fruit flies, pointing out the potential implications for Integrated Pest Management, with special reference to the development of behavior-based control tools and new tephritid lures.

Improvement of *Bactrocera carambolae* management by insect growth regulator and semiochemicals for simultaneous application of SIT and MAT

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The carambola fruit fly (CFF), *Bactrocera carambolae* Drew & Hancock is an emerging global fruit pest of economic and quarantine importance. With its polyphagous behaviour similar to that of its closely related sibling, the Oriental fruit fly (OFF), *B. dorsalis* (Hendel), this species is currently expanding beyond its native range of Indo-Malaya to the Neotropics. Thus, because of its potential global spread, CFF is a potential target for simultaneous MAT and SIT application. However, there are certain challenges pertaining to insect mass-rearing and release components for CFF-SIT program. Compared to the OFF, CFF possessed a significantly lower fecundity, fertility and a slower sexual development rate. These significantly increases the holding time and cost for maintaining both immature and mature flies in mass-rearing factory prior to release of semiochemical-treated sterile males in a SIT programme. Currently, it is not known of any effects of chemical treatment in accelerating the maturity of CFF to possibly reducing SIT cost. We seek to evaluate effects of synthetic insect growth regulator(s), i.e., methoprene and/or

semiochemical supplement to accelerate CFF sexual development without compromising its sexual performance. Due to the presence of morphological hybrids between OFF and CFF in the field-infested fruits, the first year of CRP will be dedicated to establish a pure-bred line of CFF in the laboratory to yield stable and sufficient fly colonies. Effects of insect growth regulator (IGR) treatment on male sexual behaviour, pheromone production, mating success and longevity will be investigated (years 2-3 of CRP). Effects of exposure to semiochemicals such as zingerone (ZN) to earlier sexual development and improved mating performance of *B. carambolae* will also be investigated (years 3-4 of CRP). Finally, in light of implementing a simultaneous MAT and SIT program, we will also investigate if semiochemical(s) supplement can suppress the response of CFF males to male lures such as methyl eugenol (years 4-5 of CRP). Current progress and work performed related to the CRP will be presented in this first RCM.

Cross-species comparison of fruit fly olfactory perception of host fruits as a way of exploring new attractive blends

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Semio-chemicals are used for decades as one of the tool included in integrated pest management of Tephritid fruit flies. Two main categories of fruit fly attractants are used so far: food-based lures for attract-and-kill strategies, since they target virgin females among other insects, and male lures like methyleugenol for monitoring male populations of targeted species. Food-based lure reach only a small fraction of the fruit fly population and are not very specific. Male lures are specific and effective attractants, but their simultaneous use with the release of sterile male remains hazardous, and the use of methyleugenol is forbidden in Europe due to its carcinogenicity.

La Réunion Island houses nine fruit fly species impacting fruit and vegetable production, including *Bactrocera dorsalis*, *Bactrocera zonata*, *Ceratitis capitata* and *Zeugodacus cucurbitae*, with partial host-range overlapping. We aim to identify new attractants for these species with exploring an alternative aspect of their chemical ecology: the kairomones of host fruits that attract gravid females. We explored the volatilomes of 28 different fruit species and estimated the genericity of hundreds of volatile compounds among the preferred hosts of the main fruit fly species. Besides, olfactory functions are conserved among Tephritid species due to shared evolutionary history, although specific olfactory sensitivities might emerge from specific selection pressure. We compared the olfactory sensitivity of different fruit fly species to chemical compounds released by strawberry-guava and found kairomones that are detected by conserved neuronal pathways and others that are detected by species-specific pathways. The distinction between these two categories of kairomones should be an asset in designing kairomone blends that could induce generic or specific attraction of fruit flies.

Evaluation of integrated male annihilation techniques (MAT) and sterile insect technique (SIT) to enhance the performance of sterile males of *Bactrocera* species

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Insect pests belonging to genus *Bactrocera* sp. (Diptera: Tephritidae) pose major biotic stress on various fruits and vegetable crops around the world. Diverse bacterial communities are associated with *Zeugodacus* and *Bactrocera* sp. and play an important role in the fitness of sterile insects. Understanding the gut bacterial composition is essential for the development of effective pest management strategies. We studied the gut bacteria of fruit fly species using culture dependent and next-generation sequencing (NGS) of *16S rRNA* gene. Gut bacterial isolates were further evaluated for their attractant potential to attract fruit fly adults. In culture dependent method, several bacterial isolates belonging to families Enterobacteriaceae (>75%), Bacillaceae, Micrococcaceae and Staphylococcaceae were recovered from gut of *Z. cucurbitae* and *B. dorsalis* and identified based on *16S rRNA* gene sequence analysis. Whole cell culture and supernatants of some bacterial isolates (*Klebsiella, Citrobacter* and *Providencia* species) significantly attracted *Z. cucurbitae* and *B. dorsalis* adults in laboratory and field conditions. The supernatants of these gut bacteria attracted significantly more number of females than males. We will focus on to identify the chemical composition in whole cell culture and supernants of gut bacteria using GCMS analysis. The abundant compounds (synthetic chemicals) will be checked for the response to fruit fly by electroantennography (EAG) recordings. Similarly, cucurbits, mango, sapota and guava fruits will be collected from fruit fly infested orchards and volatile present in these fruits will also be explored. The significance of volatiles compounds originating from insect gut bacteria and fruit fly's hosts in insect pest management program will be discussed.

Towards combined use of MAT and SIT for management of *Bactrocera* (Diptera: Tephritidae) fruit flies: Developing methods of delivering methyl eugenol, hormones, and diet supplements

Ihsan ul Haq

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For combined use of male annihilation technique (MAT) and the sterile insect technique (SIT), the system of delivering methyl eugenol (ME), adoptable in sterile males holding and release facilities has been remained a limiting factor. The ME application by aromatherapy which would potentially be adoptable in existing sterile males holding and release facilities enhanced the *Bactrocera dorsalis* GSS males mating success similarly as did by ME feeding. The *B. dorsalis* GSS males fed on protein diet attained sexually maturity at 6-7 days of age and topical application of juvenile hormone analogue (methoprene) did not further accelerate their sexual maturity. The ME therapeutic and protein fed 6-7 d old males achieved significantly higher mating success over non ME treated, protein fed 15d old males. These findings are encouraging and may provide the basis for combined use of MAT and SIT for management of *B. dorsalis* populations, however, some fundamental questions are needed to be answered and the following activities are planned to answer these questions during the next 18 months.
The *B. dorsalis* males once fed on ME showed very little response to ME until 20 d, however, repeat feeding response of ME-aroma treated males is not known yet and will be determined. Mating compatibility of *B. dorsalis* GSS with wild *B. dorsalis* Pakistan population will be assessed. Mating competitiveness of young males (6-7 d old) treated with ME-aromatherapy against wild males will be assessed. Over-flooding ratio of ME-aroma treated *B. dorsalis* GSS

sterile males for transferring the sterility to wild females in field cages will be determined. Effect of ME-aromatherapy on *B. dorsalis* GSS male's longevity/starvation survival and the re-mating behavior of females will be assessed. The role of protein as an adult diet is critical and the effective composition of protein diet will be identified.

Assessment of simultaneous application of SIT and MAT to enhance management of *Zeugodacus cucurbitae* (coq.) and B*actrocera dorsalis* (Hendel) in Bangladesh

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Tephritid fruit flies are the world's worst fruit pests, causing substantial hardship in developing economies. Various tephritid fruit fly pests, especially the *Zeugodacus*/*Bactrocera* species are causing damage of high economic significance in the Asia and Pacific region. In Bangladesh the Melon fly, *Zeugodacus cucurbitae* (Coq.) and the Oriental fruit fly, *Bactrocera dorsalis* (Hendel) are considered economically most important species of different fruits and vegetables causing 10-30% losses of annual production. The Sterile Insect Technique (SIT) is an environmental friendly approach used widely to combat these tephritid flies. SIT would be applied as an area-wide management of Z. *cucurbitae* and *B. dorsalis* in key fruits and vegetables growing areas of Bangladesh. Research has already been done on the development of cost effective artificial diet, optimization of quality parameters, sterility doses, field-cage trials, and area-wide population estimation of theses flies for the application of SIT. Mass trapping of *Bactrocera* fruit flies using different lures has already been conducted in mixed vegetations and selected mango orchards of Bangladesh. Gut bacterial community of major *Bactrocera* fruit flies were isolated, identified and selected bacteria spp. were used as probiotic to improve the fitness parameters of SIT targeted fruit flies. The mating enhancement of *B. dorsalis* using the Golden Shower Flowers, *Cassia fistula* (L.) also reported under laboratory condition.

The present research programme aimed to: i. Identify the effective semiochemicals/hormone as pre-release treatments and develop the effective delivery system of semoichemicals against *Z. cucurbitae* and *B. dorsalis*, ii. Determine the age of flies and time of exposure of selected semiochemicals only or in association with hormone added adult diets on different fitness parameters viz., acceleration of reproductive organs, earlier sexual maturation and male sexual performance, survival, flight ability of mass-reared and sterile flies under laboratory and semi-field conditions, iii. Detect the amount of pheromone from haemolymph/rectal gland, and iv. Evaluate the key parameters such as reduced response of exposed sterile *Z. cucurbitae* and *B. dorsalis* males to MAT traps, determine the sterile to wild over-flooding ratio to assess their influence on the effectiveness of simultaneous MAT and SIT application in semi-field cages and large field condition.

Overall, identification, appropriate application of semiochemical only or in combination of hormone/nutrient as pre-release treatments on the vital physiological and behavioural fitness i.e., early sexual development, mating competitiveness, longevity and reduce response to lure baited traps of mass reared irradiated and control fruit flies would be elucidated to improve the

efficacy of SIT as well as simultaneous application of MAT and SIT as a novel strategy for *Bactrocera* fruit fly management.

Semiochemicals for pre-release treatment of *Bactrocera dorsalis* and relative response to male lures in semi field conditions

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The study explores the potentially synergistic relationship between Male Annihilation Technique (MAT) and Sterile Insect Technique (SIT). This will be done in Kenya under semi field conditions. The relative response of *B. dorsalis* to various lures such as Cuelure and Vertlure, will be assessed with Methyl Eugenol (ME) as control. This objective will be carried out during the first 12 months of the project. However, prior to embarking on research work, we intend to boost the existing colony of *B. dorsalis* at *icipe* and construct suitable field cages for use in experiments. Results of this objective will guide further studies in the second year to determine alternative semiochemicals which can be adopted for treating sterilized *B. dorsalis* before release to minimize attraction to ME. Using the identified semiochemical above, the optimal concentration and exposition time required to treat *B. dorsalis* prior to releases will be determined in the third year, followed by assessments on relative attraction of the semiochemical preferably in field conditions in the same year. Thereafter, the ideal age of sterilized fruit flies to be released in simultaneous MAT and SIT scenarios following treatment with semiochemicals will be determined. This activity will be conducted in the fourth year and spill over into the fifth year where trials will be conducted to evaluate and fine tune findings of the five-year study. The success of the study will largely depend on obtaining a steady supply of sterile insects from our Collaborators: Kenya Agricultural and Livestock Research Organization-Biotechnology Research Institute (KALRO-BioRI, Muguga Centre, Kenya. The five-year study is expected to generate information on semiochemicals which can be fed to *B. dorsalis* to minimize attraction to ME traps, the appropriate concentrations and exposure times, as well as the ideal age of fruit flies to be fed the semiochemicals prior to release in simultaneous application of MAT and SIT in IPM programs.

Estimating the effect of the simultaneous use of male annihilation technique and sterile *Bactrocera* release on population reduction rates

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The effect of using multiple population management tools on population growth rates has been estimated for the Queensland fruit fly, *Bactrocera tryoni.* Models suggested that the simultaneous use of the male attractant, cuelure, and the sterile insect technique would be incompatible. In the model this was due to the large catch of both sterile and wild flies, but only a weekly replenishment of the sterile fly population through recurrent fly releases, in contrast to the assumed continuous recruitment of new sexually mature wild males via the maturation process. This lead to highly variable overflooding ratios, initially in favour of the sterile flies, but

quickly (1-2 days) changing to favour wild flies in the period between the weekly releases of sterile flies.

Advancements into understanding the role of male attractants on fly behavior, including the potential use of pre-feeding the attractant or related compounds to reduce male fly sensitivity to the male lure prior to fly release, may allow for compatibility of the two tools. If a reduction in the sensitivity of released flies to the lure can be realized, this would lead to the disproportionate removal of wild flies over sterile flies by MAT, thereby increasing both the overflooding ratio in favour of the sterile flies and the rate of population suppression.

Cost-effective treatments support the Male Annihilation Technique and the Sterile Insect Technique for the Establishment of a Fruit Fly Low Prevalence Area in Thailand

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The active AWIPM+SIT for fruit fly control in area of 25.9 km^2 of Trok Nong sub-district, Khlung district, Chanthaburi province, one of the marketable production areas of tropical fruits in Thailand, where *Bactrocera dorsalis* is responsible for exportation trade barrier, due to the distribution in many other varieties of soft fruits, has been supported for years and reaches a satisfactory low level of wild population. Sterile *B. dorsalis* were weekly released prior the fruits season. Male annihilation technique, the main supporting technique, integrated with bait application technique using local materials, and other environmental friendly methods were applied for population suppression. This research should help to complete the successful on exposure *Bactrocera* fruit fly especially the *B. dorsalis* and *B. correcta*, the two major insect pests of Thailand, to methyl eugenol or any other alternative semiochemicals to improve sterile male sexual performance and reduce their response to MAT traps. Since we have failed to expose the sterile *B. dorsalis* to methyl eugenol prior to release in the selected area, due to the more difficulty of delivering system to the large number of appropriate age of flies than small scale application. Besides that, core area and buffer zone of the treated area will be clearly reclassified. Trapping network and fruit sampling for surveillance system will be improved following the international standard. The sterile flies release duration will be extended longer for the appropriate sterile to wild over-flooding ratio. Simultaneous application of the SIT and MAT integrated with other compatible management practices will be correctly and intensively implemented. These all improve for the sustainability of wild target population suppression that should be the great further efforts for the cost-effective treatments for AWIPM+SIT in this area. Meanwhile improve the efficacy of *Bactrocera* fruit fly management and expand to other fruit

fly control areas in Thailand. All treatments will be applied following the International Standards for Phytosanitary Measures to lead the active AWIPM+SIT area reaches the goal and be certified as a fruit fly low prevalence area under National Plant Protection Organization certification.

Pre-release Raspberry Ketone (RK) feeding and Non-responder selection as approaches for developing cuelure non-responsive Queensland fruit flies for combined SIT and MAT

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Queensland fruit fly, *Bactrocera tryoni* (Froggatt) is a major pest of horticulture in Eastern Australia, and is a major biosecurity threat to other regions. Outbreaks in fruit fly free regions are commonly managed by the use of MAT and protein baits followed by SIT. Significant increases in the efficacy of control measures could be achieved by the simultaneous application of MAT and SIT, both because MAT devices would not need to be retrieved from the field before the application of SIT and because continued, but selective, activity of MAT devices would substantially bias overflooding ratio in favour of sterile males for any given level of release rate. Combined SIT and MAT requires techniques that yield released sterile males that have minimal attraction to cuelure but are also ecologically and sexually competent. That is, in assessing the potential use of phytochemicals or genetic techniques to reduce male response to MAT, it is important to consider the broader effects of treatments on physiology and performance.

Work published to date by our group confirms (1) suppression of cuelure response in Q-flies fed RK, (2) accelerated sexual development of RK-fed Q-flies and (3) efficacy of additional matings of young RK-fed males in terms of sexual inhibition in mates. Our unpublished, but largely complete, research confirms (4) accelerated development of reproductive organs in RK-fed Q-flies, (5) increased vulnerability to nutritional and desiccation stress in RK-fed Q-flies, (6) changes in pheromone production and release of RK-fed males, and (7) no differences between RK-fed and unfed male Q-fly in cuelure response of antennae and maxillary palps, indicating that RK-induced reduction of cuelure response is mediated centrally rather than at chemosensors in antennae and maxillary palps. Over the coming 18 months we will seek to complete and publish these studies and will continue exploring the positive and negative impacts of pre-release RK treatment on sexual and ecological competence. Field releases will be used to investigate effects of pre-release RK feeding on survival and dispersal.

In addition to continuing our studies of RK-feeding effects, we will also use standard selection techniques to establish lines of Queensland fruit fly with reduced and elevated cuelure response. If sufficiently high levels of non-response can be generated by selection then this might provide a more reliable outcome without the potential negative effects of RK-supplements on ecological competence. We will commence these studies in the coming year, and once stable lines are available we will investigate potential deficiencies in these lines to ensure that such lines are fit for use. The genetic basis of lure non-response will then be investigated through the remaining period of CRP activity in order to enable the use of targeted molecular techniques, such as CRISPR-Cas9, to knock out cuelure response. Such approaches have the potential to induce more complete effects than either RK feeding or selection.

Effect of Methyl Eugenol feeding on re-feeding and mating behaviour

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Re-feeding rate of males emerged 25 d was not significantly affected by the time (1-24 h) of feeding ME. With males feeding ME for 2 h , there was no significant difference in re-feeding rate after 15, 25, 35 d. The mating success and mating competitiveness would decrease with the increasing of males age. There was no significant influence on mating success rate of males feeding ME at different ages, but the mating competitiveness of male could be improved significantly by feeding ME .The proportion of males with non-trend to ME would increased with generations of selecting ,however, the proportion would stabilize about 30%.Results also showed that males trending to ME were significantly lower than control males since F2 generation, that had equal mating competitiveness to wild males.
Feeding ME could substantially increase the mating competitiveness of sterile males, but would not increase both the total number of multiply mating and the continuous mating frequency of sterile males. At the same time, sterile males fed methyl eugenol did not significantly affect the remating of females at 5 days after the initial mating, but did increase the remating frequency of females at 10 and 15-days after the initial mating. If both sterile males and wild males were exposed to ME together the sterile males had higher mating competitiveness than wild males.

Simultaneous application of MAT and SIT for management of B*actrocera dorsalis* in South Africa: environmental and physiological considerations

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Males of many *Bactrocera* Macquart (Diptera: Tephritidae) species are attracted to one of a number of semiochemical compounds referred to as phenylpropanoids. Due to their attractiveness, some of these chemicals, including methyl eugenol, are highly effective lures in traps that are also used for “male annihilation technique” (MAT). MAT can be used prior to sterile insect technique (SIT) programmes targeting *Bactrocera* species to reduce the abundance of wild males, thereby improving the sterile:wild male ratio. This sequential application of MAT followed by SIT was believed necessary to prevent attraction of released sterile males to MAT baits. However, it has been proposed that MAT and SIT may be applied simultaneously because prior exposure to a phenylpropanoid reduces subsequent male attraction to the same or a different chemical. An issue that needs to be addressed is whether pre-release nutritional treatments supported by joint FAO/IAEA-funded research are also compatible with simultaneous application of MAT and SIT. This is because sterile male *Bactrocera* fed yeast hydrolysate to improve their survival and mating performance respond more strongly to phenylpropanoid lures. This project, based in South Africa, will investigate some of the environmental and physiological variables that may interact with semiochemical pre-treatment to influence response of

Bactrocera dorsalis (Hendel) to methyl eugenol. Specifically, we will: (1) Identify alternative, affordable and readily available semiochemicals for pre-release treatment of *B. dorsalis* that reduce responsiveness to traps baited with methyl eugenol; (2) Establish the responsiveness of *B. dorsalis* to traps baited with methyl eugenol when pre-treated with selected semiochemicals and nutritional supplements under varying semi-field conditions; (3) Determine whether pre-release treatment of *B. dorsalis* with selected semiochemicals and nutritional supplements affects flight, dispersal and metabolic rate; and (4) Document the field response to methyl eugenol by sterile *B. dorsalis* receiving pre-release treatments relative to untreated and wild males.

Invasion of *Bactrocera dorsalis* in reunion island

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Since its detection in Kenya in 2003, *Bactrocera dorsalis* (Tephritidae), known as the "oriental fruit fly", has rapidly spread throughout sub-Saharan Africa to the islands of the Indian Ocean. In Réunion Island, this species was first observed in April 2017. This new invasion was much feared for the damage it could cause especially on host species of economic importance such as mango. It was crucial to follow the progress of the invasion and determine the range of host-plant species. We collected fruits of over one hundred species all over the island, in cultivated and no-cultivated areas, and at different altitudes, to monitor emergences of fruit flies. We replicated this sample three times, during the two austral winters following the first detection of the new fruit fly species (2018, 2019) and one summer (2019). Our first results show that, one year after the first detection of *B. dorsalis*, this species was present throughout the island, up to 1600 masl. *B. dorsalis* was found in fruits of many cultivated species such as mango, banana or papaya, sometimes with a considerable quantity of individuals per fruit (up to 200 pupae on a single mango). This species also infested invasive species such as *Psidium cattleianum* and *Syzygium jambos* and some native species such as *Aphloia theiformis*. *B. dorsalis* was observed on nearly 40 host-plant species. *Fopius arisanus*, a parasitoid of the genus *Bactrocera*, was observed emerging from a narrow range of host-plant species compared to its host and with relatively low parasitism rate (<30%). These early results are essentials to determining host plants and areas to target in control programs.

Evaluation of the feasibility of the management of the invasive fruit fly pest *Bactrocera dorsalis* on mango in Réunion using the sterile insect technique

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The Oriental fruit fly, *Bactrocera dorsalis*, a severe invasive pest of horticultural crops worldwide, has been detected in April 2017 on the island of Réunion, situated in the South West part of the Indian Ocean. A year after its first detection it caused considerable damages to mangoes, with yield losses recorded up to 90% in some orchards. Its invasion led in 2017 to the massive increase of phytosanitary products in addition to those already used in orchards. Besides, methyl eugenol-based mass trapping systems targeting males are not authorized in

European countries, so other techniques of mass trapping should be considered to reduce adult population in this tropical insular context.

We'll present the project proposal to be developed within the coming years that aims at decreasing *B. dorsalis* populations in mango orchards using a multi-scale approach. We will explore the combination of various protection practices including the eventual use of a SIT strategy. In order to optimize the use of a SIT, it is both necessary to get an extensive knowledge on the bioecology of this pest in la Réunion and to find techniques to enhance the competitivity of males of this species. The proposed project will rely on the acquisition of i) bioecological basic knowledge on *B. dorsalis* in Réunion, ii) assessment of the effect of exposure of selected *B. dorsalis* pest species to semiochemicals on earlier sexual maturation and improved male sexual performance iii), evaluation of agro-ecological practices (prophylaxis, trapping, parasitism rate ...) iv) and attracting females of *B*