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WORKING MATERIAL

Assessment of Simultaneous Application of SIT and MAT to Enhance Bactrocera Fruit Fly Management

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NOTE

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SUMMARY

The male annihilation technique (MAT) is an environmentally friendly pest control method. The 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 control method with no known negative environmental impacts, involves the mass-rearing of male insects, sterilizing them with ionizing 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 been rare and so far only 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 high 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.

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 sometimes as early adults 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.

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

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 pre-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 minimize responsiveness to traps

d. Identify alternative semiochemicals for pre-release treatment, but recognizing 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

COORDINATED RESEARCH PROJECT (CRP)

This Coordinated Research Project (CRP) is based on a Consultants Meeting on Assessment of Semiochemicals to Enhance Bactrocera spp. Sterile Male Performance that was held from 25-27 November 2015 in Vienna, Austria. The overall objective of this new **CRP D4.10.27**, approved for the **period 2018-2023**, is to explore the potentially synergistic relationship between MAT and SIT when applied simultaneously to dramatically improve the efficacy of Bactrocera fruit fly management. The assessment of semiochemicals to enhance Bactrocera spp. SIT application against these pest fruit flies will include:

1. Assessment of the effect of exposure of major Bactrocera pest species to semiochemicals on earlier sexual maturation and improved male sexual performance, as well as reduced response of exposed sterile males to MAT traps.
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 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.

SECOND RESEARCH COORDINATION MEETING (RCM)

The RCM was held virtually with 38 research contract and agreement holders, as well as observers from Australia, Bangladesh, Brazil, China, Czech Republic, France, India, Israel, Malaysia, Mauritius, New Zealand, Pakistan, South Africa, Thailand, United States of America, Viet Nam and International Centre of Insect Physiology and Ecology (ICIPE).

Twenty presentations on research progresses were delivered. The various topics in the presentations covered all the research themes of the CRP, such as Pre-release treatment methodology for reduced lure response; Effects of pre-release treatments on fly performance; Novel attractants - microbiota, cuticular lipids, and plant extracts; Selection for non-responders; Mechanisms of lure response and semi-field and field assessments of SIT +/- MAT.

The list of participants is given in Annex 2, the agenda of the meeting in Annex 3 and the abstract of the presentations are compiled in Annex 4

SITUATION ANALYSIS

Lures: Genetics, Physiology and Behavior

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. correcta*, *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 recognize 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:

Subtheme 1. Pre-release treatment methodology for reduced lure response

Subtheme 2. Effects of pre-release treatments on fly performance

Subtheme 3. Novel attractants - microbiota, cuticular lipids, and plant extracts

Subtheme 4. Selection for non-responders

Subtheme 5. Mechanisms of lure response

Subtheme 6. Semi-field and field assessments of SIT +/- MAT

Research Subthemes

Subtheme 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.

Subtheme 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.

Subtheme 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.

Subtheme 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.

Subtheme 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.

Subtheme 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.

INDIVIDUAL WORKPLANS

Contracts/Agreements List

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

	Subtheme 1. Pre-release treatment methodology for reduced lure response	Subtheme 2. Effects of pre-release treatments on fly performance	Subtheme 3. Novel attractants - microbiota, cuticular lipids, and plant extracts	Subtheme 4. Selection for non-responders	Subtheme 5. Mechanisms of lure response	Subtheme 6. Semi-field and field assessments of SIT +/- MAT
Anthony Clarke (AUL)			*B. dorsalis* *B. tryoni*		*B. dorsalis* *B. tryoni* *B. cacuminata*	
Bishwo Mainali (AUL)	*B. tryoni*	*B. tryoni*		*B. tryoni*	*B. tryoni*	*B. tryoni*
Olivia Reynolds (AUL)		*B. tryoni*			*B. tryoni*	*B. tryoni*
Mahfuza Khan (BGD)	*B. dorsalis* *Z. cucurbitae* *Z. tau** *B.zonata*	*B. dorsalis* *Z. cucurbitae* *Z. tau** *B.zonata*				*B. dorsalis*
Cristiane Ramos de Jesus (BRA)		*B. carambolae*	*B. carambolae*			
Qinge Ji(CPR)	*B. dorsalis*	*B. dorsalis*				*B. dorsalis*
Lucie Vaníčková (CZR)			*Bactrocera & Zeugodacus*			
Helene Delatte (FRA) Laura Moquet (FRA)						*B. dorsalis*
Vincent Jacob (FRA)	*B. dorsalis*		*B. dorsalis*			
Ashok Hadapad (IND)	*B. dorsalis* *Z. cucurbitae*		*B. dorsalis* *Z. cucurbitae*			*B. dorsalis*

	Subtheme 1. Pre-release treatment methodology for reduced lure response	Subtheme 2. Effects of pre-release treatments on fly performance	Subtheme 3. Novel attractants - microbiota, cuticular lipids, and plant extracts	Subtheme 4. Selection for non-responders	Subtheme 5. Mechanisms of lure response	Subtheme 6. Semi-field and field assessments of SIT +/- MAT
Yoav Gazit (ISR)		*B. zonata*		*B. zonata*		*B. zonata*
Shepard Ndlela (KEN)	*B. dorsalis*					
Alvin Hee (MAL)				*B. dorsalis*	*B. dorsalis*	
Suk Ling Wee (MAL)	*B. dorsalis* *Z. cucurbitae* *Z. tau** *B. carambolae*	*B. dorsalis* *Z. cucurbitae* *Z. tau** *B. carambolae*	*B. dorsalis* *B. carambolae*			
Nausheen Patel (MAU)	*B. dorsalis* *Z. cucurbitae* *B. zonata*	*B. dorsalis* *Z. cucurbitae* *B. zonata*				*B. dorsalis* *Z. cucurbitae* *B. zonata*
Lloyd Stringer (NZE)						*B. tryoni* *B. dorsalis* *B. zonata*
Ihsan ul Haq (PAK)	*B. dorsalis* *B. Zonata*	*B. dorsalis* *B. Zonata*				*B. dorsalis*
Christopher Weldon (SAF)	*B. dorsalis*	*B. dorsalis*				*B. dorsalis*
Suksom Chinvinijkul (THA)	*B. dorsalis* *B. correcta*	*B. dorsalis* *B. correcta*				*B. dorsalis* *B. correcta*
Sheina Sim (USA)	*B. dorsalis*	*B. dorsalis*	*B. dorsalis*		*B. dorsalis*	*B. dorsalis*
Hien Nguyen (VIE)	*B. correcta*	*B. correcta*				*B. correcta*
*** *Z. tau* species complex**						

LOGICAL FRAMEWORK

Project Design Elements	Verifiable Indicators	Means of Verification	Important Assumptions
Overall Objective: The objective of the project is to is to explore the potentially synergistic relationship between male annihilation technique (MAT) and sterile insect technique (SIT) when applied simultaneously to dramatically improve the efficacy of *Bactrocera* fruit fly management.	N/A	N/A	Member States will continue to suffer major losses to endemic and introduced insect pests. The demand for area-wide integrated insect pest management approaches, including SIT and MAT as non-polluting suppression/eradication components, continues to increase, mandating expansion and improvement in cost-effectiveness of these environment-friendly, sustainable approaches. International trade in agricultural commodities will continue to increase and be disrupted by pests requiring expensive post-harvest and quarantine measures.

Project Design Elements	Verifiable Indicators	Means of Verification	Important Assumptions
Specific Objectives:			
1. Assess the effect of exposure of major *Bactrocera* pest species to semiochemicals on earlier sexual maturation and improved male sexual performance, as well as reduced response of exposed sterile males to MAT traps.	1. Research and development focused on generating new methods of semiochemicals exposition and evaluation of its impact on improving male sexual performance.	1. Scientific reports and peer-reviewed publications.	1. Technology can be improved by reducing the response of semiochemicals pre-treated males to the MAT devices and consequently allow the integration of SIT and MAT simultaneously.
2. Evaluate 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 to determine their influence on the effectiveness of simultaneous MAT and SIT.	2. Applied research focused on the testing of the semiochimicals treatments and its impact on the sexual performance of treated males.	2. Scientific reports and peer-reviewed publications.	2. Response of semiochemicals pre-treated males to the ME and CUE dramatically reduced.
3. Evaluate in the field the simultaneous MAT and SIT within a pilot or operational setting that includes compatible management practices.	3. Evaluation of the impact of the use of SIT and MAT applied simultaneously in the field	3. Scientific reports and peer-reviewed publications.	3. MAT and SIT applied simultaneously will be more effective on fruit fly control

Project Design Elements	Verifiable Indicators	Means of Verification	Important Assumptions
Outcomes:			
1.1. Determine the minimum amount of semiochemical required by males to reduce their lure response for a significant portion of their lifetime in the field.	1. Doses responses determined at least for one semiochemical and one fruit fly species	1. Evaluation reports under field operational conditions	1. Low response to the lures in the field when insects are pre-treated with appropriated doses of semiochemiclas
1.2. Establish the best means to confirm that adequate semiochemical delivery has been achieved physiologically, including analysis of haemolymph, rectal gland contents, and pheromone composition	2. Protocols applied for the analyses of both haemolymph and, rectal glands	2. Evaluation reports under operational laboratory conditions.	2. Semiochemicals are physiologically absorbed and easy detected by using conventional laboratory protocols
1.3. Establish the best means to minimise responsiveness to traps	3 Protocols applied to deliver the appropriated doses of semiochemicals to prevent lure responde in the field	3. Evaluation reports on field trapping.	3. Use of SIT and MAT possible due to the low response to the lures in the field when insects are pre-treated with semiochemiclas
1.4. 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	4 Different semiochemicals tested and the role and function of each specific semiochemical determined	4. Different semiochemicals tested and the role and function of each specific semiochemical determined	4 The role of Alternative semiochemicals is already know then can be used in combination with conventional semiochemicals for the enhancement of MAT

Project Design Elements	Verifiable Indicators	Means of Verification	Important Assumptions
1.5. Compare the mating age and behaviour of males fed semiochemicals only or in combination with other pre-release treatments (e.g., methoprene, dietary supplements)	5 QC and field cage test protocols applied to assess the sexual behaviour for each specific age and treatment	5. Evaluation reports under operational conditions.	5. Combination of semiocemicals accelerate the male sexual maturity and enhance mating competitiveness
1.6. Determine whether pre-release treatments of fruit flies diminish their performance, such as survival, flight, dispersal, and mating ability	6 QC, field cage and open field dispersion tests and protocols applied to assess the quality and dispersal capability of treated and untreated insects	6. Scientific reports and peer-reviewed publications.	6. Application and use of semichemicals increase or does not impact on fruit flies quality
1.7. Determine the best means of semiochemical delivery that is compatible with existing fly emergence and release systems	7 Protocols for effective and practical delivering of semiochemicals determined and applied	7 Scientific reports and peer-reviewed publications.	7. Current emergence and release technology can be easily integrated with semeocemical delivering system.
1.8. Establish the relative field response of different fruit fly species to male lures using standardised protocols	8 Protocols applied and evaluated for at least 2 species and 2 different semichemicals	8 Scientific reports and peer-reviewed publications.	8 Adequate response of fruit flies to specific lures
2.1 Determine the degree of lure response of sterile flies in field cages	1 Field test conducted for at least 2 species and 2 semiochemicals	1 Scientific reports and peer-reviewed publications.	1 Results obtained in field cage experiments are representative an equal to results obtained in open field test

Project Design Elements	Verifiable Indicators	Means of Verification	Important Assumptions
2.2 Establish the sterile:wild over-flooding ratio to determine their influence on the effectiveness of simultaneous MAT and SIT. 3.1 The use of MAT only, the simultaneous MAT and SIT (sterile insect release with MAT present) and, the sequential MAT and SIT (sterile insect release after removal of MAT) with optimal pre-treatment evaluated in field conditions	2 Test conducted for at least 2 species and 2 semiochemicals 1 Adequate sterile to wild ratio for the use of MAT only, simultaneous MAT and SIT and the sequential MAT and SIT applied open field conditions for at least 1 semiochemical and 1 species determined.	2 Scientific reports and peer-reviewed publications. 1 Scientific reports and peer-reviewed publications.	2 Open field test validated in 3 scenarios, possible 1 Open field test are feasible to assess the effect a efficiency of the combination of MAT and SIT is feasible in open field tests
Outputs: 1.1 Minimum amount of semiochemical required by males to reduce their lure response determined. 1.2 Best means to confirm that adequate semiochemical delivery established 1.3 Best means to minimise responsiveness to traps established	1. Doses response determined and tested in at least 2 species. 2. Protocols applied to determine the increment of mating success and reduction on attraction of specific lure. 3 Effectiveness of semichemicals tested and validated	1. Reports and peer-reviewed publications. 2. Reports and peer-reviewed publications. 3. Reports and peer-reviewed publications.	1. Pre-treated males with semichemicals do not respond to lures in the field 2. Available semiochemicals can be used for the implementation of MAT and SIT combination 3. Sterile males responded positive to pre-treated treatments

Project Design Elements	Verifiable Indicators	Means of Verification	Important Assumptions
1.4 Alternative semiochemicals for pre-release treatment, identified	4.. Protocols developed and alternative semiochemicals assessed in at least 2 species.	4. Reports and peer-reviewed publications.	4. Alternate semicochemicals available.
1.5 Mating age and behaviour of males fed semiochemicals only or in combination with other pre-release treatments (e.g., methoprene, dietary supplements) determined	5 Protocols developed and alternative pre-release treatments assessed in at least 2 species.	5. Reports and peer-reviewed publications.	5. pre-release treatments available.
1.6 Performance of pre-release treated fruit flies, determined	6. Protocols developed and assessed in at least 2 species.	6. Reports and peer-reviewed publications.	6. Sterile insects respond to the pre-release treatments
1.7 Best means of semiochemical delivery that is compatible with existing fly emergence and release systems determined	7 Protocols developed and assessed in at least 2 species.	7 Reports and peer-reviewed publications.	7 existing fly emergence and release systems is compatible with semiochemicals delivering system
1.8 Standardised protocols for male lures field response test developed in different fruit fly species	8 Protocols developed and assessed in at least 2 species.	8 Reports and peer-reviewed publications.	8 Different fruit species respond positive to specific lures
2.1 Degree of lure response of sterile flies in field cages determined	1 Protocols developed and assessed in at least 2 species	1 Reports and peer-reviewed publications.	1 Field cage test results are representative of open field conditions

Project Design Elements	Verifiable Indicators	Means of Verification	Important Assumptions
2.2 Determination of the influence of the Sterile:wild over-flooding ratio on the effectiveness of simultaneous MAT and SIT established	2 At least 2 sterile : wild ratios assessed in large field cages in at least 2 species	2 Reports and peer-reviewed publications.	2.Field cage test results are representative of open field conditions
3.1 The use of MAT only, the simultaneous MAT and SIT (sterile insect release with MAT present) and, the sequential MAT and SIT (sterile insect release after removal of MAT) with optimal pre-treatment evaluated in field conditions	1 Protocols developed and assessed in at least 2 species	1 Reports and peer-reviewed publications.	1 Open field test are feasible to assess the effect a efficiency of the combination of MAT and SIT is feasible in open field tests
ACTIVITIES:			
1. Hold Consultants Meeting.	1. Consultants meeting held in November 2015.	1. Consultants Meeting resulted in a recommendation to use a CRP mechanism to do research on the subject.	1. Consultants meeting approved.
2. Announce project amongst established entomologists working on seasonal pests and establish CRP.	2. CRP announced and research contracts and agreements submitted, evaluated, and forwarded to IAEA committee.	2. Contracts and agreements issued.	2. Proposals submitted and approved by IAEA committee.
3. Organize first RCM to plan, coordinate and review research activities (2nd quarter 2019).	3. 1st RCM held in 2019.	3. Working material printed and distributed for 1st RCM.	3. Research activities commence. Reports published and distributed following each RCM.

Project Design Elements	Verifiable Indicators	Means of Verification	Important Assumptions
4. Carry out R&D.	4. Research carried out by contract and agreement holders.	4. Reports and publications.	4. Research activities continue, progress satisfactory.
5. Second RCM to analyse data and draft technical protocols as required (late 2020).	5.2nd RCM held in 2020.	5.Working material printed and distributed for 2nd RCM; Research published in scientific literature and disseminated to member states and scientific community.	5. Renewal requests and continued funding of RCM's and CRP.
6. In conjunction with the second RCM meeting 2-3 day hands-on workshop on "Assessment of the effect of exposure of major *Bactrocera* pest species to semiochemicals".	6. Workshop held in 2020. Harmonized procedures and trainees capable of implementing novel techniques.	6. Workshop report integrated as an appendix to the report of the Second RCM.	6. There is need for training; techniques and instructors are available.
7. Continue R&D.	7. Research carried out by contract and agreement holders.	7. Reports and publications.	7. Renewal requests and continued funding of RCM's and CRP.
8. Review the CRP after its third year.	8. Mid-CRP review carried out.	8. Report of mid-CRP review.	8. Mid-CRP review by Agency committee is positive.
9. Convene third RCM to evaluate and standardize protocols (early2022).	9. 3rd RCM held in 2022.	9. Working material printed and distributed for 3rd RCM; Research published in scientific literature and disseminated to member states and scientific community.	9. Mid-year review of CRP approved by IAEA committee. Research activities continue, progress satisfactory.
10. Continue R&D.	10. Research carried out by	10. Reports and publications.	10. Renewal requests and

Project Design Elements	Verifiable Indicators	Means of Verification	Important Assumptions
	contract and agreement holders.		continued funding of RCM's and CRP.
11. Hold final RCM to review data and reach consensus (late 2023).	11. Final RCM held in 2023.	11. Final CRP report.	11. Research and dissemination activities concluded.
12. Evaluate the CRP and submit evaluation report.	12. CRP evaluation carried out.	12. CRP evaluation report.	12. CRP evaluation by Agency committee is positive.
13. Summarize and publish advances of CRP in a series of joint publications.	13. CRP members submit papers summarizing activities.	13. Publication in scientific literature.	13. Manuscripts submitted, edited, peer reviewed and published.

PUBLICATIONS DURING THE CRP

Hiap, W.W., Wee, S.L., Tan, K.H. & Hee, A.K.W. (2019) Phenylpropanoid sex pheromone component in hemolymph of male Carambola fruit fly, *Bactrocera carambolae* (Diptera: Tephritidae) *Chemoecology* 29, 25-34.

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Rabiatul, A.S. & Wee, S.L. 2019. Zingerone improves mating performance of Zeugodacus tau (Diptera: Tephritidae) through enhancement of male courtship activity and sexual signaling. Journal of Insect Physiology 119: 103949.

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Tasnin S., Silva R., Merkel K. & Clarke A.R. (2020) Response of male Queensland fruit fly (Diptera: Tephritidae) to host fruit odours. *Journal of Economic Entomology* 113: 1888-1893.

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Akter, H., Mendez, V., Morelli, R., Pérez, J., & Taylor, P.W. (2017b) Raspberry ketone supplement promotes early sexual maturation in male Queensland fruit fly, *Bactrocera tryoni* (Diptera: Tephritidae). *Pest Management Science*, 73, 1764-1770.

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ANNEX 1: INDIVIDUAL 5-YEAR WORK PLANS

ANNEX 2: LIST OF PARTICIPANTS

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

28 June to 2 July 2021 Virtual

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ANNEX 3: AGENDA

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

28 June to 2 July 2021 (Virtual)

MONDAY, 28 JUNE 2021

13:00 – 13:10	**Rui Cardoso Pereira:** Welcome statement and goals of the meeting
13::10 – 13:30	**Lucie Vanickova:** Epicuticular chemoecology of the melon fruit fly, *Zeugodacus cucurbitae* (Diptera: Tephritidae): temporal dynamics and electrophysiological responses
13::30 – 13:50	**Laura Moquet** & **Vincent Jacob:** A multi-scale approach to optimize the application of SIT on *Bactrocera dorsalis* in mango orchards in la Réunion: bioecology, sterile male competitiveness and female attractants.
13:50 – 14:10	**Shepard Ndlela**: Potential of *Mondia whitei* (Hook.f.) (Gentianales: Apocynaceae) and selected Phenol esters in reducing responsiveness of *Bactrocera dorsalis* to methyl eugenol
14:10 – 14:30	**Nausheen Patel:** Assessing age of exposure of *Bactrocera zonata* to semiochemicals and their responsiveness to Methyl eugenol baited traps
14:30 – 14:50	**Christopher Weldon:** Simultaneous application of MAT and SIT for management of *Bactrocera dorsalis* in South Africa: environmental and physiological considerations
14:50 – 15:10	**Cristiane Ramos de Jesus:** Effect of semiochemical on carambola fruit fly behaviour, survival, and lure response to improve pest management in Brazil
15:10 – 15:30	**Discussion and next day plan**

TUESDAY, 29 JUNE 2021

09:00 – 09:20	**Mahfuza Khan:** Use of semiochemicals as prerelease supplement for management of *Bactrocera dorsalis* (Hendel) and *Bactrocera zonata* (Saunders) for simultaneous application of SIT and MAT
09:20 – 09:40	**Ashok Hadapad:** Response of fruit fly adults (*Bactrocera* and *Zeugodacus* sp.) to semiochemicals and supernatants of bacteria isolated from fruit fly's stages
09:40 – 10:00	**Yoav Gazit:** The effect of various physiological parameters on the response of *Bactrocera zonata* males to methyl eugenol
10:00 – 10:20	**Alvin Hee**: Developing non-methyl eugenol-responding male Oriental fruit flies supplemented with semiochemical exposure for improved SIT

10:20 – 10:40	**Suk Ling Wee:** Improvement of *Bactrocera carambolae* management by insect growth regulator and semiochemicals for simultaneous application of SIT and MAT
10:40 – 11:00	**Suksom Chinvinijkul:** 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
11:00 – 11:20	**Ihsan ul Haq:** Mating competitiveness, survival and repeat feeding behavior of *Bactrocera dorsalis* GSS (Diptera: Tephritidae) males treated with methyl eugenol as a pre-release treatment
11:20 – 11:40	**Discussion and next day plan**

WEDNESDAY, 30 JUNE 2021

07:00 – 07:20	**Bishwo Mainali & Ronald Lee:** Efforts toward development of stable lure-unresponsive strains in Queensland fruit fly through pre-release feeding and genetic mapping
07:20 – 07:40	**Nicholas Manoukis:** Comparison of three behavioral assays to quantify *Bactrocera dorsalis* response to ME
07:40 – 08:00	**Lloyd Stringer:** Predicting the outcomes of MAT + SIT on population suppression
08:00– 08:20	**Mohammed Abul Monjur Khan**: Raspberry ketone induces changes in the transcriptome of sterile male Queensland fruit fly: insights into factors regulating cue-lure selection
08:20– 08:40	**Peter Prentis:** Determining the heritability and genetic basis of lure responsiveness in Bactrocera fruit flies
08:40 – 09:00	**Qinge Ji:** Influence of Methyl Eugenol (ME) on Sterile Males of *Bactrocera dorsalis* (Hendel)
09:00 – 09:20	**Trang Vu:** Assessment the impact of adult diet and methyl eugenol on male sexual success of *Bactrocera correcta*
09:20 – 09:40	**Discussion and next day plan**

THURSDAY, 1 JULY 2021

08:00 – 08:30	Review of the Logical Matrix Framework
08:30 – 09:00	R&D workplans for the next 18 months and next day plan

FRIDAY, 2 JULY 2021

08:00 – 09:00	Review and finalization of the report

ANNEX 4: ABSTRACTS OF PRESENTATIONS

Epicuticular chemoecology of the melon fruit fly, *Zeugodacus cucurbitae* (Diptera: Tephritidae): temporal dynamics and electrophysiological responses

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The melon fly, *Zeugodacus cucurbitae* (Coquillett), is considered the most destructive pest of melons and other related crops. Despite its economic importance, little is known about the production and chemoreception of cuticular hydrocarbons in both sexes of *Z. cucurbitae*. Investigating the chemical ecology of this important pest may shed light on mate choice mechanisms, adding useful information to improve behaviour-based control strategies. In this research, the epicuticular composition of *Z. cucurbitae* males and females was investigated using two-dimensional gas chromatography coupled to mass spectrometric detection and interpreted using multivariance factorial analysis. A consistent differentiation of the chemical profiles over time was observed; in young individuals the chemical profiles did not differ between sexes, while sex-specific differences were highlighted in mature flies. Sexually mature males produced the 2,8-dialkyl-1,7-diaxospiro[5.5]undecane, previously reported as a female-specific pheromone component. The fly olfactory sensitivity to these compounds was explored through gas chromatography coupled to chopped three point electroantennography and electropalpography detectors. The antennae of both males and females were sensitive to three male components whereas the palps were sensitive to the female component. The responsiveness of other tephritid species was also investigated, concluding that *Z. cucurbitae* uses species-specific olfactory receptors to detect these compounds. Overall, this study adds basic knowledge to the chemical ecology of the melon fruit fly, highlighting potential implications for developing behaviour-based control tools and new tephritid lures.

A multi-scale approach to optimize the application of SIT on *Bactrocera dorsalis* in mango orchards in la Réunion: bioecology, sterile male competitiveness and female attractants.

Laura Moquet[1], Vincent Jacob[1] and Hélène Delatte[2,3]

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Our project aims at decreasing the *B. dorsalis* population in a targeted area around mango orchards in la Réunion using a multi-scale approach, including the eventual use of a SIT strategy. In order to optimise the use of a SIT, it is necessary to get an extensive knowledge on the bioecology of the pest, to find techniques to enhance the competitiveness of males and to search for female attractants.

Regarding bioecology, we study the spatial and temporal distribution of host fruits in the targeted area, the reproductive behaviours and the dispersal of wild and lab-reared individuals. (1) For one year, we collected potential host plants of *B. dorsalis* and recorded their infestation level, phenology and localisation. We found about fifteen host plants around the mango orchards. Our objective is to perform the dynamic cartography of host availability in the landscape. (2) Reproductive behaviour (number of mating, refractory period) of males and females were studied and compared between wild and laboratory-reared individuals (competitiveness). Our results show that females remated rarely and were not more attracted by wild males than by laboratory-reared males. Next, the same approach will be used to compare wild and sterile individuals. (3) Finally, to study the dispersion of *B. dorsalis* in mango orchards, we will perform mark-release-recaptures of sterile flies. This year, we received an agreement from the French administration to introduce sterile flies in La Reunion.

Regarding the enhancement of male competitiveness, (4) we will test the possibility to decrease the attractiveness of male flies toward methyleugenol traps using an aversive conditioning. Regarding female attractants, (5) we searched for volatile cues commonly emitted by the many host-fruits of *B. dorsalis* and (6) we searched, through gaz-chromatography coupled with electroantennographic detector, for compounds emitted by medium-ripe and ripe mangoes and specifically detected by *B. dorsalis* but not by other Tephritidae species. The attractiveness of candidate lures will be tested for *B. dorsalis* females.

Potential of Mondia whitei (Hook.f.) (Gentianales: Apocynaceae) and selected Phenol esters in reducing responsiveness of *Bactrocera dorsalis* to methyl eugenol

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The independent application (applied sequentially) of Sterile insect technique (SIT) and male annihilation technique (MAT) is known to be effective in suppressing pestiferous fruit fly species and their phenomenal success is documented widely. However recent concerns regarding cost and ease of application has led to calls for integration of MAT and SIT to increase efficacy (applied simultaneously). For this to be possible, released irradiated flies must have a reduced affinity for semiochemicals baited traps to ensure sustained competitiveness for mating with females against wild types. Irradiated male flies fed on methyl eugenol have been shown to exhibit enhanced mating success and reduced attractiveness to semiochemicals when released. The current focus of research is therefore to identify alternative substances which when fed to irradiated flies prior to release, increases performance and reduce their attractiveness to traps baited with methyl eugenol. We are in the

process of evaluating the possible effects of *Mondia whitei*, a shrubby woody perennial plant endemic to Africa and lauded for its potency as an aphrodisiac as well as phenol esters such as Eugenol, Vanillyacetone and Trans-cinnamic acid on the response of *Bactrocera dorsalis* when fed on for various time intervals. Laboratory feeding and wind tunnel assays show that *Mondia whitei* may reduce responsiveness of *B. dorsalis* to methyl eugenol by up to 8-fold when compared to the control in a dose dependent manner. Irradiated flies preferred feeding on 1% methyl eugenol followed by 20% Vanillyacetone and 20% Trans-cinnamic acid. Following the ingestion of the substances, mortality was significantly lower in flies that ingested the various substances, being lowest in those that ingested Vanillyacetone 5%. In the wind tunnel bioassays, there were mixed responses to methyl eugenol by flies fed on various concentrations of test substances. There is therefore need to optimise concentrations to reduce attraction to methyl eugenol. Experiments are currently ongoing.

Assessing age of exposure of *Bactrocera zonata* to semiochemicals and their responsiveness to Methyl eugenol baited traps

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Breeding of flies in large numbers and sterilizing using a radiation source for use in SIT negatively impact on flies' performance. Exposures to parapheromones have been effective in reducing these negative effects and improving flies sexual performance. Quite some investigations have been done for fruit flies in the genus *Bactrocera*, most notably *B. dorsalis* (Hendel) (Diptera: Tephritidae), on the use of chemical products commonly referred to as male lures to influence sexual performance and boost SIT. However, the age of exposure at which the flies respond most effectively is an important parameter and this has been investigated in the first part of this research relative to *Bactrocera zonata*.

Male Annihilation Technique (MAT) has been successfully used in many suppression programmes as regards to the *Bactrocera* pest species and even to eradicate isolated populations, such as on islands or during outbreaks. However, removing MAT prior to release is time consuming, labour intensive and costly. The target here is to continuously deplete fertile males from pest populations and replacing them with sterile males. However, such 'male replacement' requires a means of suppressing attraction of released sterile males to lures used in MAT. One observation done by shelly in 1994, was the reduced attraction of wild males who had access to abundant natural ME sources. Other studies of several *Bactrocera* species showed that pre-release exposure of males to plant-derived semio-chemicals and synthetic lures dramatically reduces their subsequent response to attractant lures. The second part of the research involved exposing *Bactrocera zonata* flies to Methyl eugenol by aromatherapy, and assessing their response to ME baited traps in field cages.

Results of the first part of research concludes that *Bactrocera zonata* flies can be exposed as early as two days after emergence as the semiochemical effect is not lost as in the case of *Zeugodacus cucurbitae*, but the effect is enhanced after a few days following exposure. If flies will be released on Day 7-8 as in the actual case in Mauritius, then exposing around day 5 or 6 (nearing approximate age of maturity for *Bactrocera zonata*) is recommended. Thus,

once released the flies will be more sexually competitive with an increased mating probability soon after release. This is very important given that some released flies get lost due to predation, bad weather and due to absence of food or water near release point.

In the second part of the research, the trap catches recorded were significantly different for the treated and non-treated flies for both categories of flies with a higher catch of non-treated flies in the baited trap. Reducing captures of sterile flies, as the present study shows to be possible, is likely to allow increased SIT efficiency and cost effectiveness.

Ongoing research is on deriving a proper method of semiochemical delivery to sterile flies. Different agar formulations with different concentrations of Methyl eugenol and cuelure are being tested with *Bacterocera dorsalis* and *Zeugodacus cucurbitae*. Trials are being done in field cages to assess their attractancy to pheromone baited traps following several days of agar feeding (prepared with ME and Cue) post emergence.

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

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This project, based in South Africa, aims to address some of the environmental and physiological variables that may interact with semiochemical pre-release treatments to influence response of *Bactrocera dorsalis* (Hendel) to methyl eugenol. During the reporting period we had planned to (A) Identify alternative, affordable and readily available semiochemicals for pre-release treatment of *B. dorsalis* that reduce responsiveness to traps baited with methyl eugenol, and (B) 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.

Progress towards both of these objectives has been delayed due to slow provision of customised research infrastructure (field cages), damage to field cages by storms, but to a much larger extent, due to a national lockdown in South Africa to limit the spread and deaths caused by COVID-19. However, we have been able to begin work towards Objective A. Flies from three different age groups were to be marked with fluorescent pigments (yellow, orange or red, 2g / L of pupae), and males fed with one of the four selected semiochemicals at either 0, 8 or 18 days after adult emergence. The semiochemicals that we purchased, which are readily available in South Africa, are methyl eugenol (positive control), eugenol, vanillyacetone (also referred to as zingerone) and trans-cinnamic acid (a plant precursor to methyl eugenol). Our protocol involves feeding 25 males of each age group with either 0%, 1.25%, 2.5% or 5% concentrations (in 1:3 hydrolysed yeast:sugar; total of 16 diets) of one of the semiochemicals for two days and releasing them in a field cage (one per concentration) at the Innovation Africa campus of the University of Pretoria. Each cage contains three potted citrus trees, and in each cage methyl eugenol-baited trap plus an unbaited trap (both with

insecticide) are placed in the trees. We release flies four times per day: 6:30 to 8:00, 10:00 to 11:30, 13:30 to 15:00 and 17:00 to 18:30. At the end of each period and in each cage, flies in both traps are counted and age group identified in the laboratory under a UV light. We also count number of flies that die before being released.

We planned to assay each semiochemical treatment four times but so far we have only completed one replicate with methyl eugenol. In all age groups, we observed that response to the methyl eugenol-baited trap was reduced if flies had been fed methyl eugenol before release. Only 1-7% of the flies were caught in the methyl eugenol-baited trap (0-5 % for the control trap) if they had been fed methyl eugenol prior release, and this effect was strong even at the lowest concentration of 1.25% in the adult diet. However, methyl eugenol supplementation caused a drastic increase in mortality of the 0-day age group, with 55-62% mortality in comparison with only 7% if flies were not fed methyl eugenol. The older age groups appeared to tolerate methyl eugenol supplementation better, with a mortality rate of 2-7%. So far it seems that sexually mature male *B. dorsalis* not receiving a semiochemical treatment respond to methyl eugenol-baited traps most strongly in the middle of the day.

Our future plans are to complete Objectives A and B, beginning in the Austral Spring, before proceeding to the other planned objectives to: (C) Determine whether pre-release treatment of *B. dorsalis* with selected semiochemicals and nutritional supplements affects flight, dispersal and metabolic rate; and (D) Document the field response to methyl eugenol by sterile *B. dorsalis* receiving pre-release treatments relative to untreated and wild males. This will be achieved with the help of a postdoctoral researcher, Dr Kévin Malod, who will address Objective A, a current MSc student, Ms Tania Pogue, who will work on Objective B, and the appointment of a new PhD student to work on the remaining objectives. Completion of Objective A is critical, because it underpins methodological decisions for the other objectives.

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

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The research activities scheduled for 2020 was strongly affected by the SARS CoV-2 pandemic. Embrapa, aiming to ensure the safety of its employees, adopted a home office system on March 19, 2020, and it should be valid until October 4, 2021. During this period only essential activities could be performed, such as maintenance of *B. carambolae* colony on artificial diet, preparation of both export documents and 2,000 pupae for shipment to the IPCL, and online training for volatile collection of *B. carambolae* hosts. 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.

Use of semiochemicals as prerelease supplement for management of *Bactrocera dorsalis* (Hendel) and *Bactrocera zonata* (Saunders) for simultaneous application of SIT and MAT

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The Oriental fruit fly, *Bactrocera dorsalis* (Hendel) and the peach fruit fly, *Bactrocera zonata* (Saunders) are severe invasive pests of horticultural crops worldwide including Bangladesh. Males of many *Bactrocera* species under the family Tephritidae are strongly attracted to methyl eugenol (1,2-dimethoxy-4-(2-propenyl) benzene), a naturally occurring compound. This attraction is so powerful that methyl eugenol in combination with insecticides has been successfully used in "Male Annihilation Technique (MAT)". Recently much attention has been given to the male lure, Zingerone (4-(4-Hydroxy-3-methoxyphenyl)butan-2-one) due to its ability to attract a wide variety of dacine fruit fly species. The current research findings recommend simultaneous deployment of MAT and Sterile Insect Technique (SIT) that would enable the immediate use of SIT to combat outbreaks of these fruit flies. SIT programs rely heavily on mating competitiveness and the survival of the released sterilized male flies. The mating performance of male flies is influenced by the pre-release nutritional supplements and the age of individuals. Therefore, the appropriate delivery system and the age of flies need to be take into account to study the impact of semichemicals as pre-release treatment on the acceleration of reproductive development and improvement of sexual performance as well as reduce response to traps used in MAT. The present investigation under joint FAO/IAEA granted Research Project was therefore undertaken: (i) To determine the enhanced mating success of ME aroma-treated sterile and control *B. dorsalis* under laboratory and semi-field cage; (ii) To determine the response and mating success of Zingerone/ME aroma-treated

sterile and control *B. dorsalis* and *B. zonata* under laboratory condition; (iii) To evaluate the reduce response of ME aroma-treated *B. dorsalis* to ME baited traps under semi-field cage trials; and (iv) To evaluate the influence of ME incorporated diet treatments on the reproductive organs of *B. dorsalis* and *B. zonata*.

Response of fruit fly adults (*Bactrocera* and *Zeugodacus* sp.) to semiochemicals and supernatants of bacteria isolated from fruit fly's stages

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The teprhitid fruit flies are the major pests of economically important fruit crops and vegetables. The mechanism of host plant selection by fruit flies is influenced by various volatile compounds produced by plants. Recently, it has been observed that certain gut bacteria associated with fruit fly attract the fruit fly adults. Semiochemicals like methyl eugenol and cue lures are highly effective in attracting adults of *Bactrocera* and *Zeugodacus* sp. and are widely used as male annihilation technique (MAT). In this view, response of pheromone lures (methyl eugenol and cue lure) were evaluated for *Bactrocera* and *Zeugodacus* adults trapping in Indian field conditions. Among the fruit fly species, *B. dorsalis* (87.15%) was the predominant followed by *B. correcta* (11.06%) and *B. zonata* (9.47%). In addition, we have tested some plant based essential oils for attraction of Pumpkin fruit fly (*Z. tau*) and Melon fly (*Z. cucurbitae*) adults. Clove oil and wheat germ oil attracted more *Z. tau* adults whereas peppermint oil and eucalyptus oil attracted more adults of *Z. cucurbitae*. Both males and females adults were attracted to essential oils in range of 25-80%. Over 85 gut bacteria were isolated and purified from wild males and different developmental stages of mass-reared *Z. tau* using culture dependent approach. The purified bacteria were identified using 16S rRNA sequencing and further supernatants of bacteria were tested for attractant potential of fruit fly adults. The major bacterial families like Enterobacteriaceae and Bacillaceae were the predominant in wild males and different stages of mass-reared *Z. tau*. The supernatants of purified bacteria like *Bacillus*, *Providencia* and *Citrobacter* genera are attracted both male and female adults of *B. dorsalis, Z. cucurbitae* and *Z. tau* under laboratory conditions. The response of adults of fruit fly species to lures, essential oils, gut bacteria and their importance in sterile insect technique (SIT) for the management of fruit fly species will be discussed.

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

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The attraction of the male peach fruit fly *Bactrocera zonata* (PFF) to methyl eugenol (ME) is critical for the development of control strategies that combine the sterile insect technique (SIT) and the Male Annihilation Technique (MAT). To characterize this attraction and factors affecting it, we used flies obtained from our colony in the Plant Protection and Inspections Services (PPIS) of Israel. Two main assays were developed to investigate ME-male attractiveness: a laboratory ME-response system, and a semi-field test under double-screen cages bearing natural environment of citrus orchard. The semi-field test consisted of male peach fruit flies release-recapture (in ME loaded traps) studies. These two assay systems enabled us to answer the following questions: (a) is the ME-response age dependent? The results showed that one-week-old adults showed limited response, while the older flies (5 w old), which correspond with the sexual maturation of the fly, showed a higher response rate. Two-week-old flies have an intermediate response. (b) How does larval diet affect adult attraction to ME? Adults developed in artificial larval diet showed higher ME response than adults emerging from mango and pomegranate fruits. However, exposing larvae to sub-lethal doses of ME in their diet, had no significant effect on adult response, indicating that most likely ME is not the missing factor. (c) Does irradiation affect attraction to ME? Irradiation of pupae with 100 Gy, sterilized the flies but did not affect the male response to ME. (d) How does early adult exposure to ME aroma affects post-exposure attraction? Early exposure of males to aroma of ME, significantly reduced their post-exposure response to ME. This observation can be used as a 'ME- aromatherapy' for the pre-released sterile flies, reducing their attraction to ME, and allowing the combination and coexistence of both: SIT and MAT applications. It should be noted that a considerable proportion of the males showed no reaction to the ME ("ME-indifferent males").

For the coming period of the project, we plan to:

1. Further investigate the phenomena of "ME-indifferent males" to better understand its implication: (a) Is that a local anecdote? (b) Do other flies have such "indifferent" males? (c) Are indifferent PFF males fertile and reproductive?
2. Study the effect of the larva nutrition (e.g., fruit vs. larval diet), that were found to affect ME response, on metabolites loads of lipids and proteins and on gonadal development.
   Studying the effect of mating on ME-response, and of ME exposure on sexual maturation and mating
3. Due to the results with full sterilization irradiating dose of 100 Gy in which sterilized and non-irradiated flies have the same response rate to ME, the proposal of studying sterility of sub-sterilizing doses planned in the original proposal (i.e., 20, 40 and 60 Gy), is not relevant any longer, and will not be carried out.

Developing non-methyl eugenol-responding male Oriental fruit flies supplemented with semiochemical exposure for improved SIT

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Non-methyl eugenol (ME)-responding male Oriental fruit flies (*Bactrocera dorsalis*) would enable simultaneous application of MAT and SIT. We explored the possibility of developing non-responding *B. dorsalis* by selecting from a single generation of wild male flies through repeated exposures to ME. First, we assayed wild *B. dorsalis* flies reared from infested wax apples from four different locations for their attraction to ME. Following identification and 5 generations of domestication, males from each location were exposed to ME for 30 min on 9 consecutive days between 07:30-09:00, followed by 24 h interval before the next exposure. Non-attraction of males to ME was defined as absence of attraction in at least two successive exposures. In this manner, we were able to establish two separate lines of non-ME-responding males from those locations. Second, the two non-ME-responding lines were then reared for another 10 generations and were assayed at each generation for male response to ME. In those assays, there was a gradual decrease in non-responders from *ca.* 35% initially to only *ca.* 10% after the 7th generation. Third, we used non-responders in the 10th generation as sires to initiate non-responder lines (NR). We are now maintaining colonies of those NR flies to be further tested for effects of zingerone and beta-caryophyllene consumption on mating behaviour and copulation. Fourth, we also assessed the established lines of flies for their mating competitiveness compared with control laboratory strains. There was no significant difference in the number of copulations of NR and control males. Further, after 1- and 3-days' exposure to ME the NR males did not show any significant increase in number of copulations when compared with non-ME-exposed-NR males. NR males were similar to control males in attraction to beta caryophyllene (BCP) and zingerone (ZN). Preliminary results show that NR males fed on either compound had a mating advantage over control males. A plan of action for the next 18 months in this project (behavioural, physiology, biochemistry and molecular biology) will be discussed.

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 a polyphagous fruit pest of economic importance in the Southeast region. The species has a great potential of global spread due to its invasive nature and thus, a potential target for simultaneous MAT and SIT application. The reproductive potential of CFF is significantly lower than its closely related sibling species, *B. dorsalis*. This increases holding time and maintenance cost in mass-rearing factory prior to the release of 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 first seek to evaluate effects of methoprene, a synthetic insect growth regulator to accelerate CFF sexual development without compromising its sexual performance. The progress on the effects of methoprene treatment (M^+/M^-) along with yeast/sugar (P^+/P^-) diet on sexual maturation and pheromone production will be reported. Current results showed that the inclusion of yeast diet has a profound effect while methoprene application has a negligible effect on sexual maturation of CFF. Simultaneous pheromone gland extraction has been completed but pheromone analysis could only resume after Covid-19 condition improved and laboratory reopened. In light of this outcome, a revised work plan for the next 18-month will be presented and discussed.

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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Core area and buffer zone of the three selected suitable areas to establish SIT+AWIPM pilot test were clearly classified. Trapping network and fruit sampling for surveillance system were improved following the international standard, and traps were weekly service continuously. Integrated control methods especially MAT was intensively applied every 1 trap/ 1 rai over the whole area during out of fruit season, flowering stage, fruiting stage until the minimal chemical control of any other pests. Waiting for the results of this CRP, all MAT devices were removed before sterile flies releasing which the release duration was adjusted in order to avoid the chemical application, but need to meet the appropriate sterile to wild over-flooding ratio. The sterile GSS White-thoraxed *B. dorsalis* was supported in the two of pilot areas, Trok Nong sub-district, Khlung district, Chanthaburi province and Nong Sano sub-district, Ban Kum sub-district, Mueang Phetchaburi district, and Tha Raeng sub-district, Ban Laem district, Phetchaburi province in 2021 without pre-release semiochemicals treatment. In laboratory, with realized about the practical field application, appropriate delivering system, dose and duration for exposure the appropriate age of sterile *B. dorsalis* and *B. correcta* flies

to methyl eugenol to reduce their response to MAT traps were tested. Methyl eugenol aromatherapy exposure at the rate of 0.5 ml/ 1000 flies and 1 ml/ 1000 flies for 1, 3, 5, 7, 9 hours provided non-significantly effect to 1, 2, 3, 4, 5 days old of the 21st generation of wild yellow thorax strain of *B. dorsalis* mass-reared sterile male flies mortality. ME aromatherapy exposure at 0.5 ml/ 1000 flies rate for 3 hours and 1 ml/ 1000 flies rate for 1 hour to the 4 days old of the 21st generation of wild yellow thorax strain of *B. dorsalis* mass-reared sterile male flies showed the non-significantly lower different of trap lure response in field cage condition to non-ME-expose mass-reared sterile male flies, but showed the significantly lower different to sexually mature wild male flies. While ME-exposure at 0.5 ml/ 1000 flies rate for 1 and 9 hours to the 2 days old mass-reared sterile male flies showed the non-significantly higher different of trap lure response to the non-ME-exposed mass-reared sterile male flies, even the sterile male flies showed the significantly lower different to the wild male flies after expose to ME for 1 hour.

Mating competitiveness, survival and repeat feeding behavior of *Bactrocera dorsalis* GSS (Diptera: Tephritidae) males treated with methyl eugenol as a pre-release treatment

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The genetic sexing strain, GSS (the strain enabling the separation of males at some developmental stages) which allowed releases of only males has been reported increasing the biological efficiency of the sterile insect technique (SIT) application. The white pupae Hawaiian GSS of *B. dorsalis* which enabled separation of males by color at pupal stage is available and needed for its evaluation against the wild strain for SIT application in Pakistan. The *B. doralis* males are strongly attracted to methyl eugenol (ME) and the ME has been used to lure and kill the males termed as male annihilation technique (MAT). The MAT application has been reported to suppress wild population of *B. dorsalis* wherever applied but the MAT as a standalone technique may not provide successful and sustainable control, therefore, the MAT application as a component of area-wide insect pest management (AW-IPM) alongwith the SIT application is advisable. Application of MAT and SIT has so far been sequential, where SIT was applied after suppressing the wild population by MAT application, however, simultaneous application of MAT and SIT can increase the cost effectiveness of control programs. The *B. dorsalis* males fed on ME has been reported for showing higher mating success over non ME fed males and very low tendency to repeat feeding on ME. ME application by aromatherapy which was foreseen as an adoptable method in sterile male holding and release facilities has also shown higher mating success of *B. dorsalis* GSS males, however, the survival and repeat feeding response of ME aroma-treated males was unknown. In SIT action programs, the sterile males are typically hold for 3-4 days in sterile males holding and release facilities and then released in the field. In the current study, mating compatibility of *B. dorsalis* GSS with wild strain of Pakistan was assessed and mating competitiveness of GSS males fed on ME on 5 d of age and evaluated 1 d after treatment, against full grown wild males (14-16 d old) under field cages conditions. Additionally, effect of ME treatment by feeding and aromatherapy on *B. dorsalis* GSS male's survival and repeat feeding on ME was assessed. The *B. dorsalis* GSS males were compatible for mating with

wild females. The GSS males fed on ME achieved significantly higher mating success over full grown wild males. The GSS males fed on protein diet since emergence and fed on ME on 5 d of age and then switching off the food and water showed the least starvation survival followed by ME aroma treated males and No ME treated males had the highest starvation survival, however, the ME Fed or ME aroma-treated males switched to only water or water and sugar showed similar survival as by No ME treated males. The males treated by ME aromatherapy on 5d age showed significantly higher tendency over ME fed males for repeat feeding on ME on 1,3,5, and 7 d after treatment. These results suggested to device mechanism of developing a ME delivery system adoptable in sterile males holding and release facilities.

Comparison of three behavioral assays to quantify *B. dorsalis* response to ME

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Quantifying the behavioral response (attraction) of insects to lures used in their detection and control is notoriously difficult. Many laboratory assays have been developed, but their repeatability, comparability, and relation to behavior in the field is not well understood. We compared three commonly used bioassay methods to estimate the attraction of *Bactrocera dorsalis* to the male lure methyl eugenol (ME) under controlled conditions: Y-tube olfactometer, small-cage arena, and rotating carousel field-cage. We used ME-fed and ME-naïve males of wild-type and genetic sexing strain stocks to compare the sensitivity of the assays based on expected responses. We also discuss practicality of administering different bioassays and implications of these results in the context of research on combining MAT and SIT for control of *Bactrocera* fruit flies.

Predicting the outcomes of MAT + SIT on population suppression

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The male annihilation technique, MAT is used to reduce the density of male fruit flies. However, if male flies can mate multiple times, then any male survivors that either do not interact with the MAT device or don't absorb a behaviour-limiting dose of the insecticide may be able to mate with additional female flies, therefore mitigating MAT's effectiveness. Additional tools like the sterile insect technique, SIT, can help to supress the population further and can benefit from the prior use of MAT in an area before to the release of sterile male flies. These sterile flies compete with the wild male population for mates. Because the outcome of mating success comprises aspects of probability of males and females finding

each other on the landscape as well as fitness traits, a higher overflooding ratio of sterile:wild males should result in a greater frequency of mating events occurring between sterile males and wild females, resulting in depressed population growth rates.

There are concerns that the simultaneous use of MAT + SIT will have a greater impact on the SIT flies released than on the wild population because SIT flies are released periodically, unlike the population that is presumably continuously replenishing itself, leading to poor sterile:wild overflooding ratios between SIT fly releases. Pre-feeding of lab-reared sterile males may overcome this by reducing the attractiveness of the male attractant to the sterile males. I will discuss the expected outcomes of the simultaneous use of MAT + SIT when sterile males are less responsive to the MAT devices.

Raspberry ketone induces changes in the transcriptome of sterile male Queensland fruit fly: insights into factors regulating cue-lure selection

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The Queensland fruit fly, *Bactrocera tryoni* (Froggatt), is a native Australian pest of almost all commercially grown horticultural fruiting crops, impacting domestic and international market access. Sterile male *B. tryoni*, fed as immature adults on the secondary plant compound raspberry ketone (RK), show a reduced attraction to cue-lure, a synthetic analogue of raspberry ketone used as the attractant in Male Annihilation Technique. Here we aimed to determine if the reduced attraction of RK-fed adult males to cue-lure may be a consequence of altered expression of chemoreception genes. A Y-tube olfactometer assay with RK-fed and RK-unfed sterile *B. tryoni* males tested the subsequent behavioural response of males to cue-lure. RK-fed, non-responders (to cue-lure) and RK-unfed, responders (to cue-lure) were sampled (whole heads) and gene expression compared by *de novo* RNA-seq analysis. Behavioral assays confirmed results of earlier field studies, that there is a significant decrease in attraction of RK-fed sterile males to cue-lure. RNA-seq analysis revealed that 269 genes in fly heads were differentially expressed between groups of RK-fed, cue-lure non-responders and RK-unfed, cue-lure responders. A total of 218 genes were up regulated and 51 genes were down regulated in RK-fed, cue-lure non-responders. Four chemoreceptor genes were up-regulated in the RK-fed cue-lure non-responder males; a putative gustatory receptor 39b; an odorant-binding protein 99c, isoform A; an odorant binding protein 56a; and CG4757 isoform A. The gene, pickpocket 17 isoform B was highly down regulated in RK-fed, cue-lure non-

responders. The enriched and suppressed genes reported here, shed light on the transcriptional changes that affect the dynamics of insect responses to chemical stimuli. Differentially expressed genes, identified in RK-fed and RK-unfed male flies will aid the understanding of factors possibly regulating cue-lure selection behaviour of lure-fed males and guide future functional studies on chemosensory genes.

Efforts toward development of stable lure-unresponsive strains in Queensland fruit fly through pre-release feeding and genetic mapping

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In the first RCM, we presented several laboratory and field cage studies that demonstrated how dietary supplements of raspberry ketone (RK) promoted sexual maturation of adult Queensland fruit fly (Q-fly), *Bactrocera tryoni* (Froggatt) and suppressed their responses to cuelure traps. Further to that, we present here 1) the effects of RK and methoprene feeding on field performance sterile Q-flies relative to untreated flies fed only sugar and yeast hydrolysate and 2) our ongoing effort to identify genes that control lure response through interspecific crosses between *B. tryoni* and *B. jarvisi*. Compared with untreated flies, more methoprene- and RK-treated flies were recaptured in cuelure traps to which only sexually mature males are attracted. At distances of 100 and 200 m from the release point recapture rates were higher for methoprene- and RK-treated flies than for untreated flies. However, in marked contrast to the previous studies that reported suppressed response of RK-fed flies to cuelure traps, in this study we recaptured significantly more RK-fed flies in cuelure traps compared with control flies. We will discuss the discrepancy in the effect of RK feeding on suppressing Q-flies' responses to cuelure traps and its potential as a means of enabling simultaneous applications of SIT and MAT. In parallel, to understand the molecular mechanism of lure response, we undertake an interspecific hybridisation approach to map genes that control lure response in *B. tryoni* and *B. jarvisi*. These two native Australian fruit fly species are distinct taxa in nature but hybridisable in the laboratory, making them an appealing model system for dissecting the molecular basis of divergent traits, including differences in lure response of sexually mature males: cuelure for *B. tryoni* and zingerone for *B. jarvisi*. We will provide an update on the development of laboratory lure attraction bioassays, interspecific crosses for mapping the traits, the construction of chromosome-level genome assemblies for *B. tryoni* and *B. jarvisi*, and our initial assessment of autosomal synteny between these two species. We will also discuss the prospects of harnessing the molecular information of lure attraction for developing stable lure-unresponsive SIT strains in *Bactrocera*.

Determining the heritability and genetic basis of lure responsiveness in *Bactrocera* fruit flies

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The males of several *Bactrocera* species (Diptera: Tephritidae) respond strongly and positively to a small number of secondary plant compounds, including methyl eugenol, raspberry ketone and zingerone (= male lures). Due to the attractiveness of male lures, they have been used for many decades for fruit fly surveillance and male annihilation technique (MAT) management practices. The application of MAT has often preceded the sterile insect technique (SIT) to prevent the attraction of mass released sterile males to these traps. However, if we could manipulate male attractiveness to lures this would allow for the simultaneous release of sterile males and MAT. To investigate the potential to manipulate lure attractiveness we first need to determine the heritability of the lure foraging trait in males of *Bactrocera* species. We used classical genetic selection trails to understand the heritability of lure foraging by selecting the most rapidly lure responsive males from lure-fed lines across nine generations in *B. tryoni*. In order to determine the genetic basis of the lure foraging trait, we performed RAD-sequencing to identify single nucleotide polymorphism (SNPs) in the F9 lure selected lines compared to the parental (i.e. starting) populations. The lure response trait was found to be highly heritable with the flies selected for the greater lure foraging ability siring offspring with yet greater lure foraging ability. Only a small number of SNPs were associated with an increase in lure responsiveness and included genes involved in addiction to substances, regulation of serotonin/dopamine levels and some of unknown function. This experiment has shown that we can manipulate the response of male fruit flies to lures as it has high heritability and that the lure response trait responds to selection. Future work can build upon this research to select for male lines that show decreased lure responsiveness for use in simultaneous SIT and MAT programs.

Influence of Methyl Eugenol (ME) on Sterile Males of *Bactrocera dorsalis* (Hendel)

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In order to further optimize the mass rearing technique of genetic sexing strain (GSS) of oriental fruit fly, and understand the influence of methyl eugenol on it, influence of methyl eugenol (ME) on sterile males of Bactrocera dorsalis (Hendel) was studied.

7 days after emergece the sterile males would show the trend to ME. ME- refeeding rate of 10 day-age sterile males was not significantly affected by the ME-feeding time. There was no significant difference in re-feeding rate after 2, 4, 6 days later when sterile males feeding ME for 2 hours. There was no significant influence on mating success rate of males feeding ME at different day-age, but the mating competitiveness of male could be improve significantly after feeding ME.

ME-feeding could substantially increase the mating competitiveness of sterile males, but would not increase both the total times of continuous mating and the continuous mating frequency of sterile males. At the same time, sterile males fed ME 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.

Key words: *Bactrocera dorsalis* (Hendel), sterile males, ME-feeding, mating competitiveness

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

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The Guava fruit fly *Bactrocera correcta* is a serious pest attacking many kinds of fruit in Viet Nam. The research on the sexual success in the field cage of this pest has been carried out. Obtained results showed that the male start mating from 5:45p.m to 6:14p.m, meanwhile most flies engaged in mating from 6:15p.m to 6:44p.m. The mating number of flies fed on sugar was the lowest, at 0.7 pairs and the highest was fed on protein-mixed sugar formula supplemented with ME of 5.0. pair (16.7%). The indicator of the sugar supplemented with ME diet and sugar mixed with protein diet were 1.3 and 2.3 pairs (representing 4.3% and 7.6% of the tested pair of flies, respectively).