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WORKING MATERIAL

***INTEGRATION OF THE SIT WITH BIOCONTROL FOR
GREENHOUSE AND OTHER CONFINED PEST INSECTS***

REPORT OF THE CONSULTANTS' GROUP MEETING

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1 Introduction

Greenhouses and other confined locations provide ideal conditions for the rapid build-up of pest populations as they are largely protected from predators and parasitoids. Many of these pests have been exposed to high pesticide pressure over many generations, and resistance has developed in many of them. Biocontrol agents are widely used to combat these pests, but not all are well controlled with biocontrol agents and when a pest gets out of control it has to be controlled with pesticides, which then disrupts the biocontrol and pollination.

The SIT is compatible with biological control and can complement biocontrol for some of those pests that are difficult to control, reducing crop losses and pesticide residues in food.

Augmentative biological control has historically focused mainly on crops grown in confined areas. Recently there is more attention for crops grown outside. For SIT the opposite direction can be observed, historically SIT has focused on area wide pest management in open environments, but in the framework of this CRP it will be assessing the viability of integrating SIT to control some pests in confined areas such as greenhouses.

This expert meeting has focused on identifying the constraints on biocontrol in greenhouses and candidates where SIT may be integrated and supplement the current biocontrol and made recommendations for the establishment of the CRP.

2 Working process

This describes the strategic approach adopted by the expert committee to make recommendations about whether there is a need for a CRP in this area.

2.1 Listing of pests of confined areas.

We initiated with drawing up a list of major pests in confined environment, being mainly pests in greenhouse and tunnel crops, fruiting vegetables, soft fruits, ornamentals (See Table 1). Confined pests that still represent a bottleneck and therefore a disruptor of current biocontrol schemes (due to corrective chemical sprayings with broad spectrum PPPs) or that are less likely to be tackled by biocontrol due to their biology (e.g. Lepidoptera with scattered oviposition sites of single eggs, which would require highly efficient searcher parasitoids to control them) were identified.

The group notes that there are different levels of confinement and greenhouses tend to be less confined and less technical moving to warmer climates. Moving into warmer climates metallic structures with glass and high tech climate control and lighting make room for wooden or metal structures with plastic or mesh screens over it ("screenhouses", "mesh houses", "casa malla"). These structures are often open or opened up at the (end) sides whenever ventilation is required. Therefore, the group notes that from open field to confinement is a continuum rather than an absolute separation. Moving into more confined cropping systems it is to be expected to improve the efficacy of SIT or inherited sterility techniques because there is less immigration of fertile females.

2.2 Evaluating pest species for SIT

We evaluated if SIT could be a method of control for some major pests (see Table 2). The following parameters were considered:

Table 1. List of greenhouse, storage, livestock and beehive pests

Pest scientific name	Common name of pest	Taxonomic group	Host	Vector Y/N	Established biological control	Excluded because
Greenhouse pests						
<i>Aculops</i>	Tomato russet mite		tomato	<i>Hirsutella thompsonii</i>	no	too small and arrhenotokous
<i>Anthonomus eugenii</i>	Pepper weevil	Coleoptera: Curculionidae	pepper			adult feeding damage
<i>Bactericera cockerelli</i>	Tomato psyllid	Hemiptera: Triozidae	tomato	Y		Vector
<i>Bemisia tabaci</i>		Hemiptera: Aleyrodidae	several			Vector
<i>Chrysodeixis chalcites</i>	golden twin-spot moth	Lepidoptera: Noctuidae	pepper tomato banana		no (eggs scattered)	
<i>Drosophila suzukii</i>	spotted wing drosophila	Diptera: Drosophilidae	soft fruits		no	
<i>Duponchelia fovealis</i>	Southern European marshland pyralid	Lepidoptera: Crambidae	ornamentals		Bt?	
<i>Echinothrips</i>		Thysanoptera: Thripidae	cut flowers			adult feeding damage , parthenogenic
<i>Frankliniella occidentalis</i>	wft	Thysanoptera: Thripidae	soft fruits, sweet peppers, cut flowers,			adult feeding damage , parthenogenic
<i>Halyomorpha halys</i>	brown marmorated stink bug	Hemiptera: Pentatomidae				adult feeding damage
<i>Helicoverpa armigera</i>	cotton bollworm	Lepidoptera: Noctuidae	polyphagous		Bt, Granulose virus	
<i>Leafhoppers</i>		Hemiptera: Cicadellidae	sweet peppers	y		vector
<i>Lygus hesperus</i>		Hemiptera: Miridae	cucumber			adult feeding damage
<i>Lyriomyza spp.</i>	Leafminers	Diptera: Lyriomyzidae				
<i>Planococcus citri</i>		Hemiptera: Pseudococcidae	tomato, sweet pepper, ..			parthenogenic?, male longevity 2d

Pest scientific name	Common name of pest	Taxonomic group	Host	Vector Y/N	Established biological control	Excluded because
<i>Neoleucinodes elegantalis</i>	eggplant stem borer, tomato borer	Lepidoptera: Crambidae	tomato, capsicum, egg plant			
<i>Nezara sp</i>		Hemiptera: Pentatomidae				adult feeding damage
<i>Scatella</i>	Shore fly	Diptera: Ephydriidae			yes	
<i>Sciaridae</i>	Fungus gnats	Diptera: Sciaridae			yes	
<i>Scirtothrips mangiferae</i>	blueberry leaf thrips	Thysanoptera: Thripidae	soft fruits			adult feeding damage
<i>Spodoptera frugiperda</i>	fall armyworm	Lepidoptera: Noctuidae	polyphagous		Bt?	
<i>Spodoptera exigua</i>	beet armyworm	Lepidoptera: Noctuidae	solanacea		Bt?	
<i>Trialeuroides vaporarium</i>	greenhouse whitefly	Hemiptera : Aleyrodidae	several	y	yes	
<i>Tuta absoluta</i>	Tomato leaf miner	Lepidoptera: Gelechiidae			EU yes not elsewhere	
Storage pests						
<i>Araecerus fasciculatus</i>	Coffee bean weevil	Coleoptera: Anthribidae	cassava:			adult feeding damage, digestive duct radiation sensitivity
<i>Cadra figulilela</i>	Dried fruit moth	Lepidoptera: Pyralidae				
<i>Callosobruchus chinensis</i>	Southern cowpea beetle	Coleoptera: Chrysomelidae				adult feeding damage, digestive duct radiation sensitivity
<i>Callosobruchus maculatus</i>	Cowpea beetle	Coleoptera: Chrysomelidae	pulses:			adult feeding damage, digestive duct radiation sensitivity
<i>Carpophilus dimidiatus</i>	Corn-sap beetle	Coleoptera: Nitidulidae				adult feeding damage, digestive duct radiation sensitivity
<i>Corcyra cephalonica</i>	Rice moth	Lepidoptera: Pyralidae				
<i>Cryptolestes ferrugineus</i>	Rusty grain beetle	Coleoptera: Laemophloeidae				
<i>Cryptolestes pusillus</i>	Flat grain beetle	Coleoptera: Laemophloeidae	paddy rice			adult feeding damage, digestive duct radiation sensitivity
<i>Cadra cautella</i>	Tropical warehouse moth	Lepidoptera: Pyralidae				

Pest scientific name	Common name of pest	Taxonomic group	Host	Vector Y/N	Established biological control	Excluded because
<i>Lasioderrna serricornes</i>	Cigarette beetle	Coleoptera: Anobiidae				adult feeding damage, digestive duct radiation sensitivity
<i>Liposcelis entomophila</i>	Psocid	Psocodea: Liposcelididae:				adult feeding damage
<i>Lophocateres pusillus</i>	Siamese grain beetle	Coleoptera: Trogossitidae	paddy rice			adult feeding damage, digestive duct radiation sensitivity
<i>Oryzaephilus surinamensis</i>	Saw-toothed grain beetle	Coleoptera: Sylvanidae				adult feeding damage, digestive duct radiation sensitivity
<i>Plodia interpunctella</i>	Indian flower moth	Lepidoptera: Pyralidae				
<i>Rhyzopertha dominica</i>	Lesser grain borer	Coleoptera: Bostrichidae				adult feeding damage, digestive duct radiation sensitivity
<i>Sitophilus oryzae</i>	Rice weevil	Coleoptera: Dryophthoridae	paddy rice:			adult feeding damage, digestive duct radiation sensitivity
<i>Sitophilus zeamais</i>	Maize weevil	Coleoptera: Dryophthoridae	rice			adult feeding damage, digestive duct radiation sensitivity
<i>Sitotroga cerealella</i>	Angoumois grain moth	Lepidoptera: Gelechiidae	paddy rice			
<i>Tribolium castaneum</i>	Red flour beetle	Coleoptera: Tenebrionidae				adult feeding damage, digestive duct radiation sensitivity
<i>Phthorimaea operculella</i>	Potato tuber moth	Lepidoptera: Gelechiidae	potato		Non commercial baculovirus, parasitoids?	
Animal husbandry						
<i>Musca domestica</i>	house fly	Diptera: Muscidae	poultry			adults vector or nuisance
<i>Fannia canicularis</i>	lesser house fly	Diptera: Fanniidae	poultry			adults vector or nuisance
<i>Alphitobius diaperinus</i>	darkling beetle	Coleoptera: Tenebrionidae	poultry			adults vector or nuisance
<i>Hydrotaea aenescens</i>	black garbage fly	Diptera: Muscidae	poultry			adults vector or nuisance
<i>Dermestes maculatus</i>	hide beetle	Coleoptera: Curculionidae	poultry			adults vector or nuisance
<i>Ornithonyssus sp.</i>	fowl mite	Mesostigmata: Macronyssidae	poultry			adults vector or nuisance
<i>Dermanyssus galinae</i>	chicken mite	Mesostigmata: Dermanyssidae	poultry			adults vector or nuisance

Pest scientific name	Common name of pest	Taxonomic group	Host	Vector Y/N	Established biological control	Excluded because
<i>Cimex lenticularius</i>	bed bug	Hemiptera: Cimicidae	poultry			adults vector or nuisance
<i>Menopon gallinae</i>	chicken lice	Phthiraptera: Menoponidae	poultry			
<i>Musca domestica</i>	house fly	Diptera: Muscidae	swine			adults vector or nuisance
<i>Haematopinus suis</i>	Hog louse	Phthiraptera: Haematopinidae	swine			adults vector or nuisance
<i>Stomoxys calcitrans</i>	stable fly	Diptera: Muscidae	swine			adults vector or nuisance
	ticks		cattle			adults vector or nuisance
	fly		cattle			adults vector or nuisance
	lice		cattle			adults vector or nuisance
	fleas		cattle			adults vector or nuisance
Beehive pests						
<i>Aethina tumida</i>	Small hive beetle	Coleoptera: Nitidulidae	bees	AFB vector		
<i>Galleria mellonella</i>	wax moth	Lepidoptera: Pyralidae	bees		good hive management	
<i>Achroia grisella</i>	lesser wax moth	Lepidoptera: Pyralidae	bees		good hive management	
<i>Varroa destructor</i>	varroa mite	Mesostigmata: Varroidae	bees	y		adults nuisance, arrhenotokous

Potential candidates for SIT

Table 2. List of species selected as potential candidates for SIT

Pest scientific name	Common name pest	Taxonomical group	Distribution	Host	Vector Y/N	Established BC	Comments	SIT existing	Mass rearing	Economic importance	References
<i>Greenhouse pests</i>											
<i>Chrysodeixis chalcites</i>	Golden twin-spot moth	Lepidoptera: Noctuidae	EU, MEA, new US, CAN	Solanaceae, Ornamentals, Cucurbitaceae, Rosaceae		Bt, Podisus, Trichogramma	seperate eggs	no data (<i>T.ni</i>)		2	
<i>Drosophila suzukii</i>	Spotted wing drosophila	Diptera: Drosophilidae	EU, US, S Am	Soft fruits		no		Part. bio radiation		1	
<i>Tuta absoluta</i>	Tomato leafminer	Lepidoptera: Gelechiidae	S Am, EU, India	Solenaceae		EU yes not elsewhere		No SIT, yes bio-radiation		EU 3, L AM 2, US 1, AS 2	Cagnotti et al 2012
<i>Neoleucinodes elegantalis</i>	eggplant stem borer, tomato borer	Lepidoptera: Crambidae	C (Mexico) - S Am	Tomato, capsicum, eggplant		no	female longevity of 6d	no data	YES	L AM 2, rest 1	Talekar et al 2002; Blackmer 2001
<i>Helicoverpa armigera</i>	cotton bollworm	Lepidoptera: Noctuidae	EU, MEA, S-Am, AS, OC	Polyphagous		Bt, Granulose virus		SIT and bio-radiation			Ocampo 2001; Pransopon et al 2000
<i>Spodoptera frugiperda</i>	fall armyworm	Lepidoptera: Noctuidae	Am (cont)	Polyphagous		Bt, Baculovirus, EPN, Telonomus remus		No SIT, yes bio-radiation			Dos Santos et al 2009; Polanczyk et al 2000; Van lenteren & Bueno 2003
<i>Spodoptera exigua</i>	beet armyworm	Lepidoptera: Noctuidae	Global except S-Am	Solanaceae, Ornamentals, Cucurbitaceae, Rosaceae		Bt, NPV		SIT and bio-radiation		1	Debolt & Wright 1976
<i>Duponchelia fovealis</i>	Southern European marshland pyralid	Lepidoptera: Crambidae	MEA, S US, CAN (ONT), EU,	Ornamentals, Solenaceae, Ornamentals, Cucurbitaceae, Rosaceae		Bt, EPN, Trichogramma, Soil mites		no data			
<i>Liriomyza</i> spp.	Leafminers	Diptera: Agramyzidae	Global	Solenaceae, Ornamentals, Cucurbitaceae		Diglyphus	short male longevity	SIT and bio-radiation			Kaspi & Parrella 2006; Walker 2012

Pest scientific name	Common name pest	Taxonomical group	Distribution	Host	Vector Y/N	Established BC	Comments	SIT existing	Mass rearing	Economic importance	References
Storage pests											
<i>Cadra figulilela</i>	Dried fruit moth	Lepidoptera: Pyralidae					SIT potential not applied	Bio-radiation	YES		
<i>Corcyra cephalonica</i>	Rice moth	Lepidoptera: Pyralidae					SIT potential not applied	Bio-radiation	YES		
<i>Cadra cautella</i>	Tropical warehouse moth	Lepidoptera: Pyralidae					SIT potential not applied	Bio-radiation	YES		
<i>Ectomyelois ceratoniae</i>	Carob moth	Lepidoptera: Pyralidae		Date			SIT potential not applied	Bio-radiation	YES		
<i>Phthorimaea operculella</i>	potato tuber moth	Lepidoptera: Gelechiidae		Potato, Solanaceae		No commercial baculovirus, parasitoids, SCLP		Bio-radiation	YES		Saour & Makee 2009
<i>Plodia interpunctella</i>	Indian flower moth	Lepidoptera: Pyralidae					SIT potential not applied	Bio-radiation	YES		
<i>Sitotroga cerealella</i>	Angoumois grain moth	Lepidoptera: Gelechiidae		Paddy rice			SIT potential not applied	Bio-radiation	YES		
Animal husbandry											
<i>Aethina tumida</i>	Small hive beetle	Coleoptera: Nitidulidae		Bees	AFB vector	EPN	80 Gy; longevity of 8d	Bio-radiation			Downey et al 2015; Schaefer et al 2010

Targeted candidates for SIT

- a. Pest distribution (continent/countries),
- b. Host range (major family of confined crops attacked),
- c. Biological control methods used and their effectiveness,
- d. Vector species yes or no?
- e. Reproduction (sexual-arrhenotokous-thelytokous),
- f. Plant parts attacked: marketable or non-marketable part,
- g. Availability of information on mass rearing,
- h. Availability on radiation biology or SIT data.

We took also into account the risk of pests invading new areas and the limitations to the use of the available natural enemies outside of their current range because of regulatory restrictions. Vectors of viruses or pests with a parthenogenetic reproduction were automatically discarded of the list because SIT will amplify the problem in the first case and for the second case SIT will be difficult to implement for species that do not rely on sexual reproduction. We ranked species from 1 to 3 in function of their economic importance based essentially on a, b, c and f criteria (1 = high economic importance; 2 = medium economic importance and 3 = poor economic importance). The final choice of potential candidates has been based on a discussion integrating economic importance and c, g and h criteria.

3 Recommendation on storage pests

The expert group recognized that among the discussed stored product pests the lepidopteran species appear to be suitable candidates for SIT/ inherited sterility. These species are generally easy to mass produce; in fact some are already mass produced as hosts and food for biocontrol agents. In addition, for most species information on the radiation biology is already available. Despite these potential opportunities, this technique has not been taken up widely for stored product pests. The expert group consists of experts on plant pests only and considers the stored products out of their expertise. For a critical review of the potential of SIT against stored product pests in confined areas the current expert group advises to consult other experts. These experts may be found from within the IOBC working group: integrated protection of stored products (https://www.iobc-wprs.org/expert_groups/11_wg_stored_products.html). First questions these experts should consider is: why the potential for SIT/ inherited sterility has not been taken up.

4 Recommendation on apicultural pests

The expert group identified a single species that might be of interest for SIT in apiculture. This species being the small hive beetle *Aethina tumida*. Radiation biology for this species is known from Downey et al 2015 and Schaefer et al 2010. However, the expert group does not consider apiculture as part of their expertise. For a critical review of the potential of SIT against this species the expert group advises to consult experts on apiculture, preferably from an area where this species is present, such as North America (http://www.uoguelph.ca/canpolin/About/a_research.html for potential contacts).

5 Recommendation on livestock pests

The expert group has considered several species of pest to livestock in confined areas as stables and barns. Although livestock is not within the expertise of the expert group, no potential candidates for

SIT could be identified. All the species that were discussed are either vectors of diseases or are a nuisance. Therefore the release of large numbers of individuals, although sterilized, is considered undesirable.

6 Identified plant pests with SIT potential in confined cropping systems

Through the procedure described above, the expert group identified three groups of pests that show potential for SIT integration.

6.1 *Drosophila suzukii*

Drosophila suzukii (Diptera: Drosophilidae) is an exotic pest of stone fruits and berries that has recently invaded Europe (Italy, France, Belgium, Austria,...), North America (United states and Canada) and south America (Brazil). This species has now a worldwide distribution (Cini et al. 2012; Asplen et al. 2015). This pest attacks a wide range of soft fruits with preference for blueberry, strawberry and raspberry (Bellamy et al.2013), crops that can be grown in confined cropped systems. The female flies lay eggs under the skin of maturing fruits and the developing larvae feed on the fruit tissues, thereby provoking the fruit to collapse.

This pest is of economic importance because when left uncontrolled the flies can cause complete loss of the harvests. Currently the control relies mostly on the application of chemical insecticides that need to be applied a few days before the fruits are harvested and may cause a threat for the health of human consumers. In addition specific cultural practices such as mass trapping, netting and strict hygiene are being used. Research on natural enemies (predators and parasitoids) is ongoing, but no biological control solutions are readily available (Cuthbertson et al. 2014; Asplen et al. 2015; Renkema et al. 2015; Stacconi et al. 2015).

Radiation biology experiments are ongoing on *D. suzukii* in collaboration with FAO/IAEA, and several universities and research institutes. Artificial rearing diets for laboratory rearing are available in literature (Chabert et al. 2013) and at least two laboratories are conducting research on mass rearing under the Suzukill project that is a multidisciplinary and international research project funded by both the French ANR and the Austrian FWF (<https://suzukill.univ-rennes1.fr/>). In addition, the IAEA has had recurrent requests from member countries about developing SIT for *D. suzukii*. The experts group has some reservations about the feasibility of SIT for this pest considering the high fecundity of this pest and the recurrent immigration of flies into the crops that, often, are not completely confined. Therefore, we recommend the involvement of an expert on the modelling of the population dynamics.

6.2 Spodoptera and Helicoverpa group

The expert group considered the *Spodoptera exigua*, *S. frugiperda* and *Helicoverpa armigera* species together as they share a similar biology. All three species are known as pests of both outdoor and important greenhouse crops such as tomato, peppers and eggplant. Biocontrol of these species relies on egg-parasitoids such as *Trichogramma* sp. or *Telenomus* sp. that are often insufficiently effective because of the short timespan to parasitize the eggs (Jarjees & Merritt, 2004). Also, the commercially available *Bacillus thuringiensis* strains appear to be insufficiently effective (Moar et al 1995; Polanczyk et al 2000; Omoto et al 2015).

For each of these species, SIT for area wide pest management has been developed in the past (Debolt & Wright, 1976; Ocampo, 2001; Carpenter et al., 1983; Carpenter et al., 1985; Carpenter et al., 1986; Carpenter et al., 1992; Hamm & Carpenter 1997; Pransopon et al 2000). However, these were never operational. The reasons why are not clear to the group and need to be looked into. The group does feel there are opportunities for SIT against these pests in confined area's that should be investigated.

Because of the past work on SIT, data on the rearing of these species and the radiation biology is available (Snow et al 1970; Snow et al 1972; Carpenter et al., 1997; Ramos Ocampo & Leon 2002; Merx-Jacques & Bede 2005; Abbasi et al 2007). This would allow the research to quickly focus on demonstrating efficiency in the confined cropping systems. As the SIT for Lepidoptera usually relies on F₁ sterility, a certain degree of damage needs to be tolerated. For fruit crops this tolerance is expected as the caterpillars primarily feed on the leaves, not on the fruits. On the other hand, the F₁ sterility will result in increased numbers of sterile eggs in the crop. These eggs will improve the efficiency of egg parasitoids if these were to be combined with the SIT. If crop damage cannot be tolerated, full sterility (95-99%) rather than F₁ sterility can be used, but this reduces the efficiency of the control as high doses are required to reach full sterility.

Because of the similarities in the biology of these three species, a CRP that coordinates the research and allows for exchange of the results is expected to lead to strong synergisms.

6.3 *Tuta and Neoleucinodes group*

Tuta absoluta (tomato leaf miner) and *Neoleucinodes elegantalis* (eggplant stem borer or tomato borer) are two emerging pests of Solanaceous crops of South-American origin (EPPO, 2005). *Tuta absoluta* has currently spread eastward into Europe as far as India and northward up to Mexico (Desneux et al. 2010). Following its introduction into Europe, North Africa and the Middle East, *T. absoluta* has already caused extensive economic damage (Tropea Garzia et al. 2012). The impact of the pest includes severe yield loss reaching 100%, increasing tomato prices, bans on the trade of tomato including seedlings, an increase in synthetic insecticide applications, disruption of integrated management programmes of other tomato pests, and an increase in the cost of crop protection. Considering its high biotic potential, its ability to adapt to various climatic conditions and the speed with which it has colonized Europe and North Africa, the potential invasion of African and especially Asian tomato crops by *T. absoluta* will probably impact heavily on the livelihood of local tomato growers and tomato agribusinesses in these regions. *Tuta absoluta* in Europe is currently sufficiently controlled by the predatory mirid bugs *Nesidiocorus tenuis* and *Macrolophus pygmeus* (Molla et al. 2009; Urbaneja et al. 2009). However, these invertebrate biocontrol agents, native to Europe, will not be an option for control when the pest reaches N. America or Asia, which is outside of the natural enemies' native ranges. Control of the pest in South America is currently based largely on chemical control. Therefore, development of a SIT for *Tuta absoluta* could provide a sustainable alternative. Radiation biology data for *T. absoluta* suggest doses of 200–250 Gy could be used to induce inherited sterility in *T. absoluta* males (Cagnotti et al. 2012).

Neoleucinodes elegantalis (tomato borer or eggplant stem borer) is a major pest of tomatoes and other Solanaceous fruit crops (e.g. *Solanum melongena* and *Capsicum* sp.) which occurs in South and Central America (Diaz Montilla et al. 2013). *Neoleucinodes elegantalis* is absent from other regions, but is considered a threat due to the importance of tomato and other Solanaceous fruit crops in

many other regions. It has been intercepted several times by the Netherlands (1 interception in 2009 and 3 in 2012) during import inspections of eggplant from Suriname and control of passenger baggage at Schiphol airport. Consequently, it has been added to the EPPO Alert List.

The objective of developing a SIT program for *T. absoluta* and *N. elegantalis* would be twofold; firstly providing a more sustainable control method for currently invaded areas where biocontrol is not yet developed and secondly providing an eradication method for these Solanaceous pests ready to use immediately a new area is invaded.

7 Irradiation

Application of SIT requires an irradiation system. Currently, the available irradiators of a suitable size for research, development and small scale production include both gamma and X irradiators.

Gamma irradiators have the advantage of reliability as the source, whilst constantly decaying, is always present and there is little to go wrong with the simple operating mechanism. Their disadvantages include the stringent regulatory, safety and security requirements for large radioactive sources, the potential risks associated with radiation and the need for periodic replenishment of the source. They can provide high dose rates (up to 200 Gy.min⁻¹) but small self-shielded machines can only provide good dose uniformity to small volumes (typically little more than one litre per load). Small research irradiators are cheaper than equivalent X ray systems but the price rises quickly for larger processing volumes.

In contrast, X irradiators can be switched off, making transport easy, the regulatory burden much lighter, the risk associated with the radiation is much lower and there is no source to replenish. But these systems are much more complex requiring sophisticated maintenance and reliable power supply. X irradiators provide lower dose rates than gamma irradiators, typically around 10 Gy.min⁻¹ but to a larger volume per load (up to 18 litres) with good dose uniformity. Suitable X irradiators for SIT are still relatively new and so far have not yet shown themselves sufficiently reliable to be adopted in operational programmes. Improved quality control and reliability, rapid service response and availability of spare parts will be critical to the future success of these systems. X irradiators are generally rather more expensive than simple gamma irradiators, but could become more competitive if any unit established a market position. The absence of any radioactive material makes these systems more acceptable to the public.

The limited number of small, self-shielded irradiation units currently available and the cost of new units pose a risk to the development and small scale application of nuclear techniques in this field. For larger scale production irradiation services can be purchased from commercial irradiator operators, but the minimum practical dose from a multipurpose irradiator, the longer exposure times and poor control of dose and dose uniformity make this less satisfactory for SIT.

8 Conclusions and Recommendations

After literature review, knowledge exchange and extensive discussions, the expert group has produced a list of key pests that could be good candidates for SIT in confined cropping systems. Also, the group recommends contacting experts on storage pests and beehive pests to have a better idea of the SIT potential for these groups. The expert group recommends having a co-ordinated research project (CRP) and drafted a proposal for the three groups of pests identified: *Drosophila suzukii*,

Spodoptera/Helicoverpa and *Tuta/Neoleucinodes*. In view of the focus on greenhouse pests the proposed title is “Integration of the SIT with Biocontrol for Greenhouse Insect Pest Management”.

9 References

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10 Annexes

Annex 1: List of participants

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Annex 2: Agenda

Monday 14 March [M0E58]

08:00 – 09:00		Registration (Bring passport / ID papers and collect security pass from Gate 1 on arrival)
09:00 – 09:30	Andrew Parker	Welcome Introduction of the participants Selection of meeting chairman and rapporteur Background and objectives of the consultants group meeting
09:30 – 10:00	Bashur Bashur	<i>The IAEA Programme of Coordinated Research Activities</i>
10:00 – 10:30		Coffee break
10:30 – 11:15	Annabelle Firlej	<i>Title to be announced</i>
11:15 – 12:00	Tom Groot	<i>On augmentative biocontrol and SIT</i>
12:00 – 13:30		Lunch
13:30 – 14:15	Lieselot van der Veken	<i>Options from biological control for pest control; experiences, prospects and regulatory framework</i>
14:15 – 15:00	Wang Shaoli	<i>Occurrence and Control of Insect Pests on greenhouse vegetables in China</i>
15:00 – 15:30		Coffee break
15:30 – 16:15	David Opatowski	<i>Feasibility study for the implementation of leafminer (Liriomyza spp.) SIT combined with biological control under greenhouse conditions in Israel</i>
16:15 – 17:00		Discussion of presentations

Tuesday 15 March [A2172]

08:30 – 10:30	Group discussions. <i>Suggested topics:</i> <ul style="list-style-type: none">• Discussion of presentations• Constraints to biocontrol in greenhouses and confined areas• Poorly controlled pests• New and invasive pests• Opportunities for integration of SIT
10:30 – 11:00	Coffee break
11:00 – 12:30	Group discussions continued
12:30 – 13:45	Lunch
13:45 – 15:15	Group discussions continued

15:15 – 15:45 **Coffee break**

15:45 – 17:00 CRP proposal design

Wednesday 16 March [A2172]

08:30 – 10:30 Identification of areas of research

10:30 – 11:00 **Coffee break**

11:00 – 12:30 Identification of areas of research continued

12:30 – 13:45 **Lunch**

13:45 – 15:15 Group discussions

15:15 – 15:45 **Coffee break**

13:45 – 17:15 Decision on meeting recommendations regarding requirements, scope and objectives for a CRP.

19:30 – **DINNER**

Thursday 17 March [A2172]

09:00 – 10:30 Drafting of CRP proposal

10:30 – 11:00 **Coffee break**

11:00 – 12:30 Drafting of CRP proposal

12:30 – 13:45 **Lunch**

13:45 – 15:15 Drafting of CRP proposal

15:15 – 15:45 **Coffee break**

15:45 – 17:00 Preparation of logical framework

Friday 18 March [M0E59]

09:00 – 10:30 Identification of potential contract and agreement holders

10:30 – 11:00 **Coffee break**

11:00 – 12:30 Drafting of meeting report

12:30 – 13:45 **Lunch**

13:45 – 15:15 Drafting of meeting report

15:15	–	15:45	Coffee break
15:45	–	17:00	Presentation of Meeting Conclusions and Outcome
17:00			End of the meeting

Title of CRP

Integration of the SIT with Biocontrol for Greenhouse Insect Pest Management

Brief Summary

This CRP will develop and evaluate SIT for insect pests in greenhouses. The CRP will focus on three groups of species that are disruptive to current biocontrol practices, or are expected to become serious pest when invading new areas. The three groups are *Drosophila suzukii*, *Helicoverpa/Spodoptera* spp. and *Tuta absoluta/Neoleucinodes elegantalis*. The background information required for SIT, such as mass rearing and radiation biology, has been developed for these greenhouse pests to a variable extent. However, for all of these groups the application in confined areas still needs to be refined and evaluated.

1. Background Situation Analysis (Rationale/Problem)

a. Current situation

Greenhouses and other confined locations provide ideal conditions for the rapid build-up of pest populations as they are largely protected from predators and parasitoids. Many of these pests have been exposed to high insecticide pressure over many generations and resistance has developed in many of them. Biocontrol agents are widely used to combat these pests, but not all are well controlled with biocontrol agents and when a pest gets out of control it has to be controlled with pesticides, which then disrupts other biocontrol and pollination.

The SIT is compatible with biological control and can complement biocontrol for those pests that are otherwise difficult to control, reducing crop losses, pesticide residues in food and risk to workers.

Augmentative biological control has historically focused mainly on crops grown in confined areas. Recently there is more attention for crops grown outside. For SIT the opposite direction can be observed: historically SIT has focused on area wide pest management, but with this CRP SIT will now enter confined areas such as greenhouses.

b. *Drosophila suzukii*:

Drosophila suzukii (Diptera: Drosophilidae) is an exotic pest of stone fruits and berries that has recently invaded Europe (Italy, France, Belgium, Austria,...), North America (United States and Canada) and South America (Brazil). This species now has a worldwide distribution (Cini et al. 2012; Asplen et al. 2015). This pest attacks a wide range of soft fruits with preference for blueberry, strawberry and raspberry (Bellamy et al. 2013), crops that can be grown in confined cropped systems. The female flies lay eggs under the skin of maturing fruits and the developing larvae feed on the fruit tissues thereby causing the fruit to collapse.

This pest is of economic importance because when left uncontrolled the flies can cause complete loss of the harvests. Currently the control relies mostly on the application of chemical insecticides that need to be applied a few days before the fruits are harvested and may cause a threat for the health of human consumers. In addition specific cultural practices such as mass trapping, netting and strict hygiene are being used. Research on natural enemies (predators and parasitoids) is ongoing,

but no biological control solutions are readily available (Cuthbertson et al. 2014; Asplen et al. 2015; Renkema et al. 2015; Stacconi et al. 2015).

Radiation biology experiments are ongoing on *D. suzukii* in collaboration with FAO/IAEA, and several universities and research institutes. Artificial rearing diets for laboratory rearing are available in the literature (Chabert et al. 2013) and at least two laboratories are conducting research on mass rearing under the Suzukill project that is a multidisciplinary and international research project funded by both the French ANR and the Austrian FWF (<https://suzukill.univ-rennes1.fr/>). In addition, the FAO/IAEA has had recurrent requests from member countries about developing conventional SIT for *D. suzukii*.

c. Spodoptera and Helicoverpa group

Spodoptera exigua, *S. frugiperda* and *Helicoverpa armigera* (Lepidoptera: Noctuidae) share a similar biology. All three species are known as pest of both outdoor crops and of important greenhouse crops such as tomato, peppers and eggplant. Biocontrol of these species relying on egg-parasitoids such as *Trichogramma* sp. (Hymenoptera: Trichogrammatidae) or *Telenomus* sp. (Hymenoptera: Scelionidae) are often insufficiently effective because of the short timespan to parasitize the eggs (Jarjees & Merritt, 2004). Also, the commercially available *Bacillus thuringiensis* strains appear to be insufficiently effective (Moar et al 1995; Polanczyk et al 2000; Omoto et al 2015).

For each of these species, SIT for area wide pest management has been developed in the past (Debolt & Wright, 1976; Ocampo, 2001; Carpenter et al., 1983, 1985, 1986, 1992; Hamm & Carpenter 1997; Pransopon et al 2000). However, these were never operationalized.

Because of the past work on SIT, data on the rearing of these species and the radiation biology is available (Snow et al 1970; Snow et al 1972; Carpenter et al., 1997; Ramos Ocampo & Leon 2002; Merckx-Jacques & Bede 2005; Abbasi et al 2007). This will allow the research to quickly focus on demonstrating efficiency in greenhouses. Because the SIT for Lepidoptera normally relies on F₁ sterility, a certain degree of damage needs to be tolerated. For fruit crops this tolerance is expected as the caterpillars primarily feed on the leaves, not on the fruits. On the other hand, the F₁ sterility will result in increased numbers of sterile eggs in the crop. These eggs will improve the efficiency of egg parasitoids if these were to be combined with the SIT. If crop damage is not tolerable, full sterility can be considered but the high doses necessary reduce the efficacy of the control.

Because of the similarities in the biology of these three species, a CRP that coordinates the research and allows for exchange of the results is expected to lead to strong synergisms.

d. Tuta and Neoleucinodes group

Tuta absoluta (tomato leaf miner) (Lepidoptera: Gelechiidae) and *Neoleucinodes elegantalis* (eggplant stem borer or tomato borer) (Lepidoptera: Crambidae) are two emerging pests of Solanaceous crops of South-American origin (EPPO, 2005). *Tuta absoluta* has currently spread eastward through Europe as far as India and northward up to Mexico (Desneux et al. 2010). Following its introduction into Europe, North Africa and the Middle East, *T. absoluta* has already caused extensive economic damage (Tropea Garzia et al. 2012). The impact of the pest includes severe yield loss reaching 100%, increasing tomato prices, bans on the trade of tomato including seedlings, an increase in synthetic insecticide applications, disruption of integrated management programmes of other tomato pests, and an increase in the cost of crop protection. Considering its

high biotic potential, its ability to adapt to various climatic conditions and the speed with which it has colonized Europe and North Africa, the potential invasion of African and especially Asian tomato crops by *T. absoluta* will probably impact heavily on the livelihood of local tomato growers and tomato agribusinesses in these regions (BBC 2016). *Tuta absoluta* in Europe is currently sufficiently controlled by the predatory mirid bugs *Nesidiocorus tenuis* and *Macrolophus pygmeus* (Heteroptera: Miridae) (Molla *et al.* 2009; Urbaneja *et al.* 2009). However, these invertebrate biocontrol agents, native to Europe, will not be a control option when the pest reaches North America or Asia, which are outside of the natural enemies' native ranges. Control of the pest in South America is currently based largely on chemical control. Therefore, development of SIT for *Tuta absoluta* could provide a sustainable alternative. Radiation biology data for *T. absoluta* suggest doses of 200–250 Gy could be used to induce inherited sterility in *T. absoluta* males (Cagnotti *et al.* 2012).

Neoleucinodes elegantalis (tomato borer or eggplant stem borer) is a major pest of tomatoes and other Solanaceous fruit crops (e.g. *Solanum melongena* and *Capsicum sp.*) which occurs in South and Central America (Diaz Montilla *et al.* 2013). *N. elegantalis* is absent from other regions, but is considered a threat due to the importance of tomato and other Solanaceous fruit crops in many other regions. It has been intercepted several times by the Netherlands (1 interception in 2009 and 3 in 2012) during import inspections of eggplant from Suriname and control of passenger baggage at Schiphol airport. Consequently, it has been added to the EPPO Alert List.

The objective of developing a SIT program for *T. absoluta* and *N. elegantalis* is twofold; firstly providing a more sustainable control method for currently invaded areas where biocontrol is not yet developed and secondly provide an eradication method for these Solanaceous pests in the event they invade new areas.

2. Overall Objective

The overall objective is to advance development and implementation of SIT for integration with other biocontrol in greenhouses.

3. Specific Research Objective (Purpose)

- To adapt SIT and inherited sterility for *Spodoptera/Helicoverpa* species for confined cropping systems
- To develop SIT for *Drosophila suzukii*
- To develop SIT and inherited sterility for *Tuta absoluta* and *Neoleucinodes elegantalis*

4. Expected Research Outputs

1. Survey on factors inhibiting the adoption of SIT and inherited sterility for *Spodoptera/Helicoverpa* group
2. Feasibility study on SIT and inherited sterility for *Spodoptera/Helicoverpa* group in confined cropping systems
3. Radiation biology for *D. suzukii*
4. Sexing system for *D. suzukii*
5. Mass rearing for *D. suzukii*
6. Feasibility study for *D. suzukii* in confined cropping systems
7. Radiation biology *T. absoluta* and *N. elegantalis*

8. Sexing system for *T. absoluta* and *N. elegantalis*
9. Mass rearing for *T. absoluta* and *N. elegantalis*
10. Feasibility study for *T. absoluta* and *N. elegantalis* in confined cropping systems

5. Expected Research Outcomes

Outcome 1: SIT and inherited sterility techniques for the targeted pest species ready for implementation in confined cropping systems

Outcome 2: SIT and inherited sterility techniques for the targeted pest species adopted in confined cropping systems

6. Relationship to Sub-programme objective and other Agency Programmes and Sub-programmes

- IPCS develops and promotes the use of nuclear techniques for the control of insect pests.

7. Action Plan (Activities)

Activity 1. Submit CRP proposal.

Activity 2. Announce project to MS and amongst established entomologists, biocontrol and pest control specialists and commercial glasshouse growers.

Activity 3. Organize first RCM to plan, coordinate and review research activities

Activity 4. Carry out R&D.

Activity 5. Second RCM to analyse data and draft technical protocols as required

Activity 6. Hold workshop on "Insect mass rearing", in conjunction with second RCM.

Activity 7. Continue R&D.

Activity 8. Review the CRP after its third year.

Activity 9. Convene third RCM to evaluate and standardize protocols.

Activity 10. Hold workshop on "Irradiation and dosimetry", in conjunction with third RCM.

Activity 11. Continue R&D.

Activity 12. Hold final RCM to review data and reach consensus.

Activity 13. Evaluate the CRP and submit evaluation report.

Activity 14. Summarize and publish advances of CRP in a series of joint publications (special issue of a scientific journal).

8. Inputs

1. Duration: 5 years.
2. Number of RCMs: 4.
3. Contracts and agreements:
 - Technical contract: 1 (1 year)
 - Agreement holders: 9.
 - Research contract holders: 9.

4. Workshops:
 - Insect mass rearing for pests of confined cropping systems (*D. sukuzii*) and irradiation protocols (with 2nd RCM)
 - Insect mass rearing for pests of confined cropping systems (Lepidoptera) and irradiation protocols (with 3rd RCM)
5. Publication of results (special journal issue).
6. Staff travel of SS to RCMs

9. Assumptions

- Continued relevance of SIT for *Drosophila sukuzii*, *Tuta absoluta*, *Neoleucinodes elegantalis* and *Spodoptera/Helicoverpa* species.

10. Foreseen Participants

Countries with significant greenhouse industries

Developed countries with capabilities for SIT or greenhouse crop research

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Developing countries with capabilities for SIT or greenhouse crop research

Country	Institute	Contact	
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Thailand	Irradiation for Agricultural Development Dividion	Watchreeporn Orankanok	watchreeporn@doae.go.th

11. Links to Technical Cooperation Projects

There are no links to current TC projects.

12. Logical Framework

Project Design Elements	Verifiable Indicators	Means of Verification	Important Assumptions
<p>Overall Objective:</p> <p>To advance development and implementation of SIT and inherited sterility for integration with other biocontrol for greenhouse and other confined arthropod pests</p>	N/A	N/A	Non-SIT biocontrol is not sufficiently controlling the targeted pests in confined cropping systems
<p>Specific Objective:</p> <ol style="list-style-type: none"> 1. To adapt inherited sterility or SIT for <i>Spodoptera/Helicoverpa</i> species for confined cropping systems 2. To develop SIT for <i>Drosophila suzukii</i> 3. To develop inherited sterility or SIT for <i>Tuta absoluta</i> and <i>Neoleucinodes elegantalis</i> 	<p>Techniques advanced</p> <p>Network established</p>	<p>Reports and publications of techniques</p> <p>Number, expertise and geographic distribution of applicants</p>	<p>Regulatory requirements permit the use of inherited sterility</p> <p>Suitable participants apply to join the CRP with a broad range of expertise</p> <p>User community is engaged</p> <p>Radiation services and insects colonies are available</p>
<p>Outcomes:</p> <ol style="list-style-type: none"> 1. SIT and inherited sterility techniques for the targeted pest species ready for implementation in confined cropping systems 			R&D has resulted in a functional SIT package for some of the targeted species

Project Design Elements	Verifiable Indicators	Means of Verification	Important Assumptions
2. SIT and inherited sterility techniques for the targeted pest species adopted in confined cropping systems	Crop losses	National statistics	Growers are willing to adopt the developed technology Growers acceptance of limited crop damage from F ₁ sterility No other sustainable control method will become available
Outputs: 1. Survey on factors inhibiting the adoption of inherited sterility for <i>Spodoptera/Helicoverpa</i> group 2. Feasibility study on inherited sterility and SIT for <i>Spodoptera/Helicoverpa</i> group in confined cropping systems 3. Radiation biology for <i>D. suzukii</i> 4. Sexing system for <i>D. suzukii</i> 5. Mass rearing for <i>D. suzukii</i> 6. Feasibility study for <i>D. suzukii</i> in confined cropping systems 7. Radiation biology <i>T. absoluta</i> and <i>N. elegantalis</i> 8. Sexing system for <i>T. absoluta</i> and <i>N. elegantalis</i> 9. Mass rearing for <i>T. absoluta</i> and <i>N. elegantalis</i> 10. Feasibility study for <i>T. absoluta</i> and <i>N. elegantalis</i> in confined cropping systems	Survey conducted Research conducted Protocols Protocols Manuscripts drafted New facts and refined understanding Protocols Test conducted Manuscripts drafted New facts and refined understanding	RCM report Research reports RCM report RCM report Manuscripts submitted Papers published, contract reports, CRP review RCM report Test reports Manuscripts submitted Papers published, contract reports, CRP review	Industry engagement Viable opportunities are identified New techniques are appropriate Techniques developed Manuscripts accepted End users engaged Techniques developed End users engaged Manuscripts accepted End users engaged

Project Design Elements	Verifiable Indicators	Means of Verification	Important Assumptions
	Recommendations for future work	RCM report	Validation will not be completed within the CRP period New opportunities identified as a result of the CRP Project is still relevant at the end of the CRP
Activities: 1. Submit CRP proposal. 2. Announce project to MS and amongst established entomologists, biocontrol and pest control specialists and commercial glasshouse growers 3. Organize first RCM to plan, coordinate and review research activities 4. Carry out R&D. 5. Second RCM to analyse data and draft technical protocols as required 6. Hold workshop on " <i>Insect mass rearing for pests of confined cropping systems (D. suzukii) and irradiation protocols</i> ", in conjunction with second RCM. 7. Continue R&D. 8. Review the CRP after its third year.			Project is approved

Project Design Elements	Verifiable Indicators	Means of Verification	Important Assumptions
<p>9. Convene third RCM to evaluate and standardize protocols.</p> <p>10. Hold workshop on <i>“Insect mass rearing for pests of confined cropping systems (Lepidoptera) and irradiation protocols”</i>, in conjunction with third RCM.</p> <p>11. Continue R&D.</p> <p>12. Hold final RCM to review data and reach consensus.</p> <p>13. Evaluate the CRP and submit evaluation report.</p> <p>14. Summarize and publish advances of CRP in a series of joint publications (journal special issue).</p>			

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Annex 4 Presentations

BERRIES PEST MANAGEMENT IN QUEBEC

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Institut de recherche et de développement en Agro-environnement



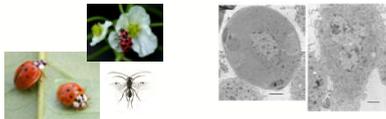
PLAN

- Very short review of professional background and of past research
- Review of recent research on *Drosophila suzukii*



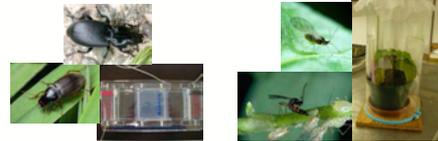
EDUCATIONAL BACKGROUND

- Master of Biology (University of Quebec at Montreal)
 - Selection of an artificial diet and artificial egg laying for a Miridae predator of *Tetranychus urticae* Koch (Firlej et al. 2002)
- Ph.D. of Biology (University of Quebec at Montreal)
 - Interaction between the parasitoid *Dinocampus coccinellae* and *Harmonia axyridis* Pallas and *Coleomegilla maculata* lengi Timberlake (Firlej et al. 2005, 2007, 2010, 2012)

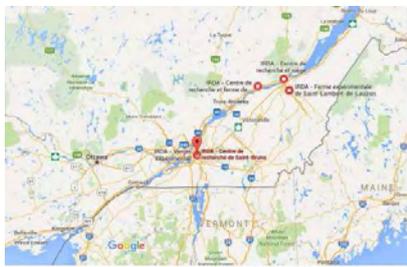


EDUCATIONAL BACKGROUND

- Post-doctoral study (FQRNT funded, University of Montreal)
 - Impact of climate change (CO₂) on interaction between aphids and parasitoids
 - Studying impact of climate change (temperature) on development rate of aphids
 - Development of molecular gut content analysis for studying control of soybean aphid by carabid beetles (Firlej et al. 2012 ; 2013)



IRDA



Entomology
Plant pathology
Weed management
Water management
Air Quality
Effluent Management.....



- Applied research
- Experimental orchard
- 90ha of organic certified crops
- High tunnel for small fruits
- Grant from Ministry of agriculture with project oriented to specific problematic



PROJECTS

1. Improving pesticide application method for cranberry production (2 years-2014-2015)
2. Selection of low-risk insecticides to control the cranberry weevil in cranberry farm (2 years-2015-2016)
3. Improved molecular identification technique of pests to meet the diagnostic needs of the agricultural sector in the context of climate change (2 years-2016-2018)
4. Creation of integrated fruit production poster for small-fruit (1 year-2016)
5. Adaptation of phytosanitary measures for pests and diseases of fruit crops in regard to climate change impacts (3 years-2016-2019)

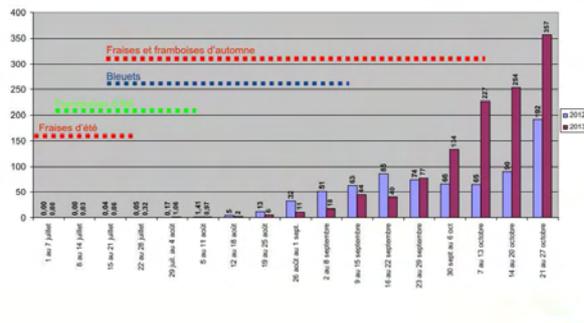


SWD IN QUEBEC



- Arrival in 2010: strawberry, raspberry, blueberry...
- Sanitation and short interval between harvest
- Insecticides: max of 10 applications in some crops (Exirel, Success 480 SC, Entrust SC, Delegate WG, Imidan 70WP instapak, Mako, Malathion 85 E)
- Harvest 3 days after insecticide application
 - Health concern
 - Damage uncontrolled
 - Few alternative methods
 - Growers discouraged
 - Sector really open to new solution

SEASONAL ABUNDANCE OF SWD



(Courtesy of C. Lacroix)

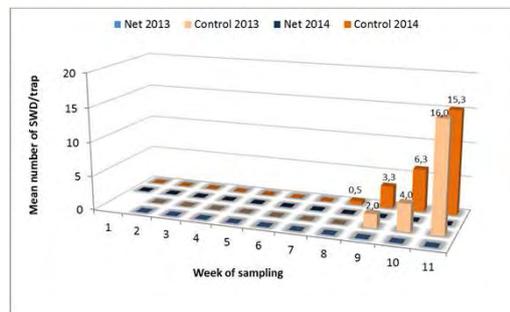
PROJECTS ON DROSOPHILA SUZUKII

1. Net exclusion to control the SWD (2 years-2013-2014)
2. Baits for SWD mass-trapping (1 year-2014)
3. The sterile insect release as a control method for SWD (3 years-2014-2017, with 1 year extension)
4. Repellents against SWD in raspberry fall (2 years-2016-2017)
5. Study of the link between the populations of spotted wing drosophila, damage and yield losses (2 years-2016-2018)
6. Literature review on SWD (1 year-2016)



PROJECTS

1. Net exclusion to control the SWD (2 years-2013-2014)



No effect on other pests and diseases
 No effect on sugar rate
 No effect on yield
 But method not yet adopted

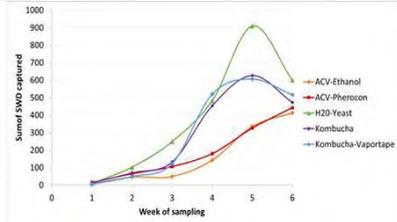
(Cormier et al. 2015)

PROJECTS ON *DROSOPHILA SUZUKII*

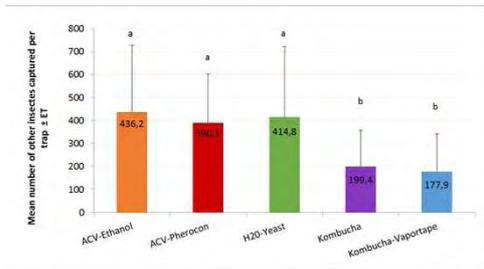
1. Net exclusion to control the SWD (2 years-2013-2014)
2. Baits for SWD mass-trapping (1 year-2014)



SELECTION OF BAITS FOR SWD MASS TRAPPING



SELECTION OF BAITS FOR SWD MASS TRAPPING



Unfortunately project of mass trapping refused by ministry...



PROJECTS ON *DROSOPHILA SUZUKII*

1. Net exclusion to control the SWD (2 years-2013-2014)
2. Baits for SWD mass-trapping (1 year-2014)
3. The sterile insect release as a control method for SWD (3 years-2014-2017, with 1 year extension)



- Since 2005 project on onion fly SIT in Quebec
- About 30 millions of flies released each year
- Facilities: company Phytodata



SIT APPLIED TO SWD

- Objectives:
 1. Developing dose-response
 - Emergence, malformation
 - Longevity
 - Egg hatching
 - Fertility
 2. Competitivity of irradiated male
 - Are they able to mate the same number of females than wild strain?
 - How much time a female will wait to mate another time after an irradiated male?
 - Which male the female will choose?
 - Field cage experiment

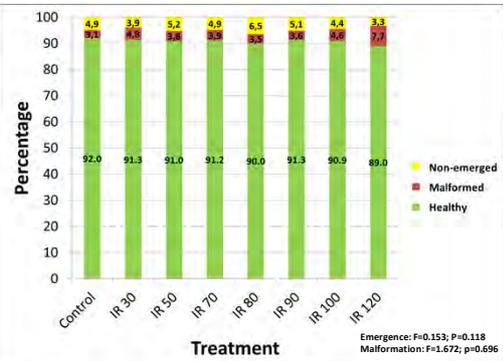


DOSE-RESPONSE

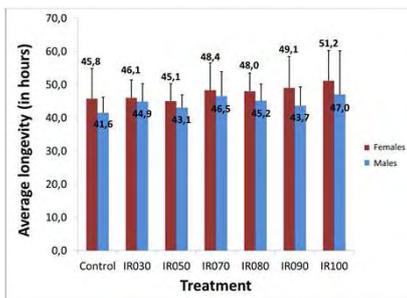
- 0, 30, 50, 70, 80, 90, 100 and 120 Gy
- Five day-old pupae
- >10 000 pupae



EMERGENCE-MALFORMATION

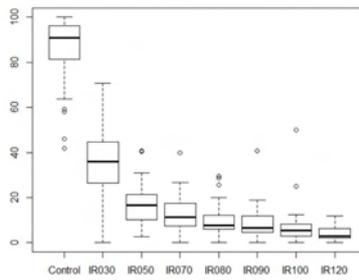


LONGEVITY



Flies fed only with water, statistics to be done...

EGG HATCHING



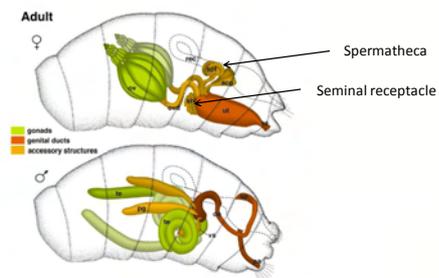
Discussion about testing higher doses

STERILITY

Treatment	Mean number of eggs/day
Irradiated females + unirradiated males	
0 Gy	39,29 ± 32,06
030 Gy	1,72 ± 2,01
050 Gy	0,03 ± 0,10
070 Gy	0,01 ± 0,07
080 Gy	0,72 ± 2,63
090 Gy	0,16 ± 0,70
100 Gy	0,00 ± 0,00
Virgin females	11,26 ± 18,91
Irradiated males + unirradiated females	
0 Gy	39,29 ± 32,06
050 Gy	35,89 ± 31,15
070 Gy	41,27 ± 28,40
080 Gy	38,22 ± 29,35
090 Gy	42,40 ± 32,21

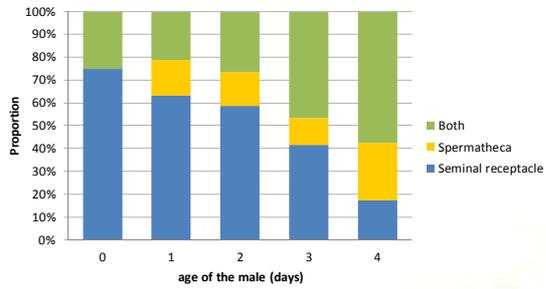
To be done:
1-Developing dose-response Complete doses
Survival of F1 larvae
2-Evaluation of irradiated male competitiveness

PRE-TEST FOR NEXT EXPERIMENTS

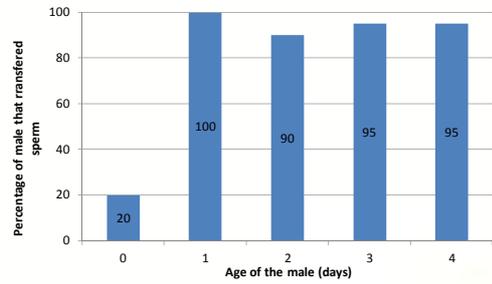


(Drawing: Voller Hartenstein, 1993)

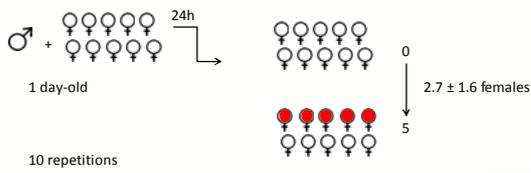
WHERE THE FEMALE STOCK SPERM?



WHEN THE MALE IS ABLE TO MATE?



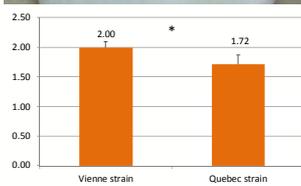
HOW MUCH FEMALES A MALE CAN INSEMINATE?



Quebec and Vienna strain



PUPAL WEIGHT



PROJECTS ON DROSOPHILA SUZUKII

1. Net exclusion to control the SWD (2 years-2013-2014)
2. Baits for SWD mass-trapping (1 year-2014)
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6. Literature review on SWD (1 year-2016)



QUESTIONS??





KOPPERT
BIOLOGICAL SYSTEMS



On augmentative biocontrol and SIT
Tom Groot, Manager R&D Entomology



KOPPERT
BIOLOGICAL SYSTEMS

OUTLINE OF MY TALK

- Current position: Koppert
- PhD work: University of Amsterdam
- In between: de Groene Vlieg

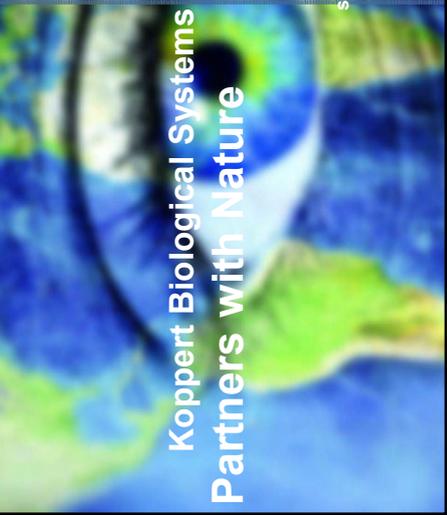


KOPPERT
BIOLOGICAL SYSTEMS



ABOUT KOPPERT

- Founded 1967
- Family company
- Head office: Berkel en Rodenrijs
- 250 people in NL, 1100 globally
- Products are sold in >120 countries



KOPPERT
BIOLOGICAL SYSTEMS

MISSION

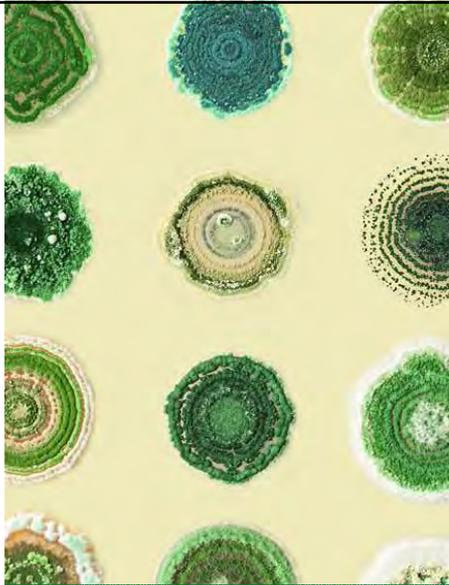
Koppert Biological Systems Partners with Nature

Koppert Biological Systems contributes to better health of people and the planet.

In partnership with nature, we make agriculture healthier, safer and more productive.

We provide an integrated system of specialist knowledge and natural, safe solutions that improves crop health, resilience and production.

3 MAIN AREAS

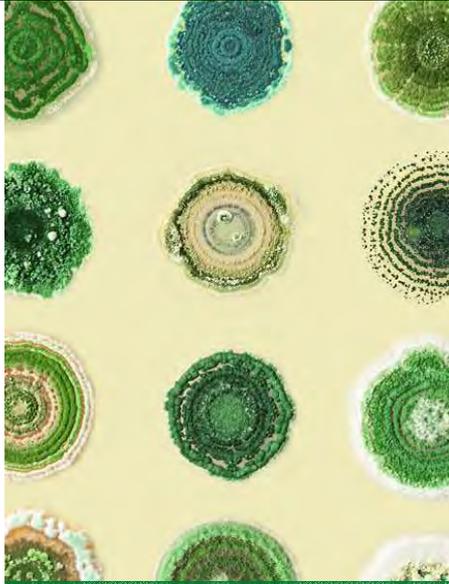


1: Microbiology

Natugro:

- Beneficial micro-organisms
 - Fungi
 - Bacteria
- Biostimulants
 - Amino, humic & vulvic acids
 - Seaweed extracts
 - Vitamins

3 MAIN AREAS



1: Microbiology

(micro)biological biocontrol

- Entomopathogenic nematodes
- Entomopathogenic fungi

3 MAIN AREAS



2: Pollination

- Mostly by bumblebees
- Main production and R&D in Slovakia
- Local production with local species
- Have paved the way for biocontrol

3 MAIN AREAS



3: Macrobials/ Entomology

Production

- Major product group of Koppert
 - Production mostly in NL
 - Sales worldwide: logistics!
 - Wide range of beneficials
 - Predatory mites
 - Predatory insects
 - parasitoids
 - And their prey/ hosts!
- cost efficiency + reliability + quality**

3 MAIN AREAS

KOPPERT
BIOLOGICAL SYSTEMS



3: **Macrobials/ Entomology**
R&D Entomology

- In NL: 8 researchers and 5 assistants
- (field) research in Spain, France, Brazil, Mexico, VS, ...
- 2016: Move to brand new R&D centre

3 MAIN AREAS

KOPPERT
BIOLOGICAL SYSTEMS



3: **Macrobials/ Entomology**
R&D Entomology

- Customer Sales
 - New biocontrol agents
 - New uses of existing BA
 - New application methods
- Customer Production
 - Rearing methods for new species
 - New rearing technologies
 - Including logistics
 - Assist with troubles

PhD work

University of Amsterdam

Supervisor:
Hans Breeuwer

Promotors
Steph Menken
Maus Sabelis

KOPPERT
BIOLOGICAL SYSTEMS



THE EFFECTS OF
SYMBIOT INDUCED
HAPLOID THELYTOKY
ON THE EVOLUTION OF
BREVIPALPUS MITES

Thomas V.M. Groot

THE EFFECTS OF
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Thomas V.M. Groot

MITES

Related to the spiders
Small
Very diverse
Receive not enough attention!



BREVIPALPUS MITES

False spider mites
Herbivores, highly polyphagous (sub-)tropical
Small
Generation 1 month
Pest: direct and virus vector



THE EFFECTS OF SYMBIOT INDUCED HAPLOID THELYTOKY ON THE EVOLUTION OF BREVIPALPUS MITES



Thomas V.M. Groot

THE EFFECTS OF SYMBIOT INDUCED HAPLOID **THELYTOKY** ON THE EVOLUTION OF BREVIPALPUS MITES

THELYTOKY

Thelytoky = virgin (asexual) reproduction producing females

NO thelytoky: virgin producing a son!



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BIOLOGICAL SYSTEMS

THE EFFECTS OF SYMBIONT INDUCED HAPLOID THELYTOKY ON THE EVOLUTION OF BREVIPALPUS MITES



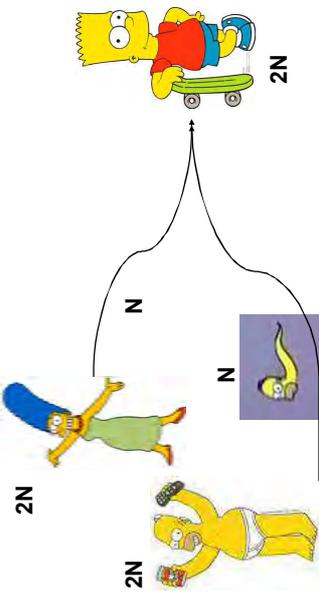
Thomas Y.M. Groot

THE EFFECTS OF SYMBIONT INDUCED HAPLOID THELYTOKY ON THE EVOLUTION OF BREVIPALPUS MITES

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HAPLOID

Most animal species are diploid; a copy of each gene from each parent.



2N

N

N

2N

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HAPLOIDIE

Diploid: 2N
 Polyploid: >2N
 Haploid: N

Males of some insect and mite species are haploid
 Females are always diploid or poly-ploid
Except for Brevipalpus!!!

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THE EFFECTS OF SYMBIONT INDUCED HAPLOID THELYTOKY ON THE EVOLUTION OF BREVIPALPUS MITES



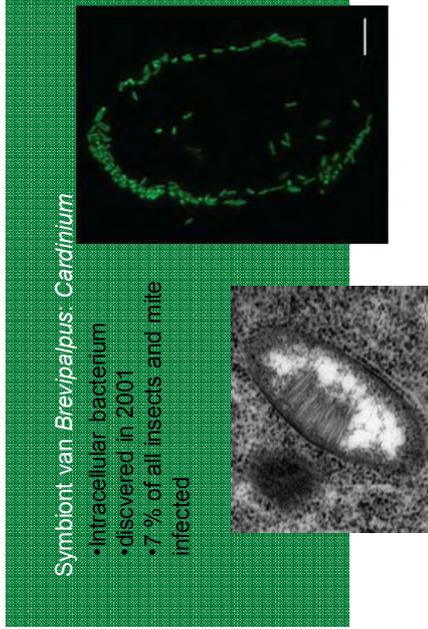
Thomas Y.M. Groot

THE EFFECTS OF SYMBIONT INDUCED HAPLOID THELYTOKY ON THE EVOLUTION OF BREVIPALPUS MITES

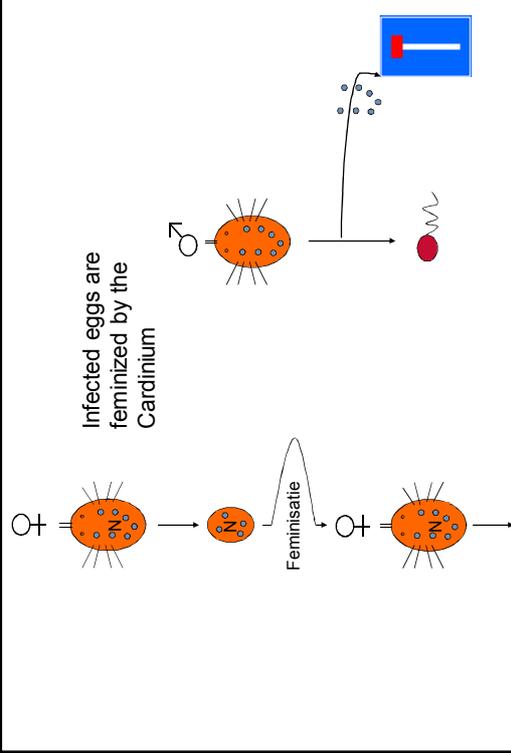
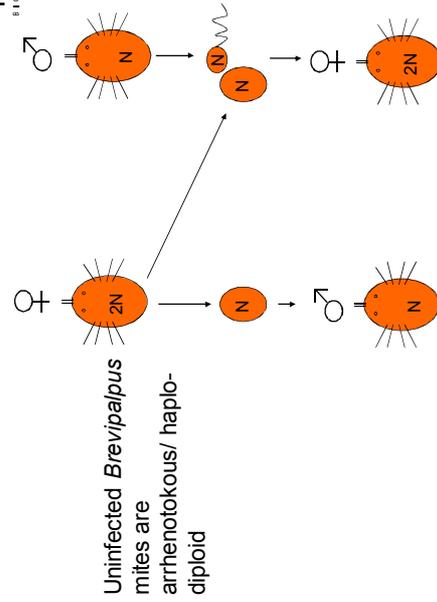
SYMBIONT

Symbiont van *Brevipalpus*: *Cardinium*

- Intracellular bacterium
- discovered in 2001
- 7% of all insects and mite infected



DE EFFECTEN VAN SYMBIONT GEÏNDUCEERDE HAPLOÏDE THELYTOKIE OP DE EVOLUTIE VAN *BREVIPALPUS* MIJTEN



DE EFFECTEN VAN SYMBIONT GEÏNDUCEERDE HAPLOÏDE THELYTOKIE OP DE EVOLUTIE VAN BREVIPALPUS MIJTEN

THE EFFECTS OF SYMBIONT INDUCED HAPLOID THELYTOKY ON THE EVOLUTION OF BREVIPALPUS MITES



Thomas V.M. Groot

EFFECTS

Asexual reproduction: Mother and daughters are all identical

Sexual reproduction: every individual inherits a unique combination of paternal genes: **VARIATION!**



MAIN CONCLUSIONS

Despite asexuality, remarkable amount of variation

Symbionts are capable of horizontal transfer

Different species became asexual at different times

Occasional males behave functional, but fail to transmit their genes

WHY MOVE FROM ACADEMIA TO COMPANY?

Preference for applied work: far more rewarding if the work evidently helps people

Don't like / good at bureaucracy

Don't like the pleading for money



Sterile Insect Technique against onion fly

T.V.M. Groot, M. Loosjes & T.C. Everaarts



de Groene Vlieg: the Green Fly

Founded in 1980

Main products

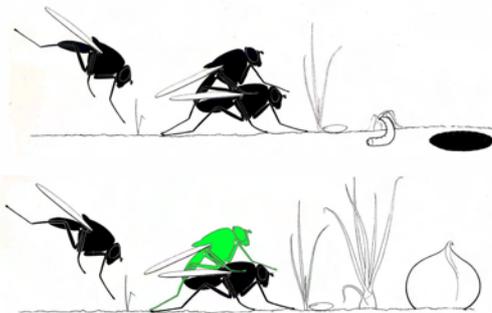
- SIT against onion fly (*Delia antiqua*)
- Supervised control of carrot fly (*Psila rosae*)
- Soil sampling for plant parasitic nematodes

Currently 100 employees



Onion fly biology

- 2 to 3 generations
 - First flight: May - 1/2 June
 - Second flight: 1/2 July - August
 - Third flight:
 - Diapauses as pupae in soil
- Host plants
 - Onions
 - Leek



Onion seedlings are eaten ...

... as are larger plants...



...and damage can be quite severe.





Producing sterile flies

- Total production: 1.000.000.000
- Produced all year round
- In summer: weekly batches taken from storage
- Sterilization as pupae
- Released on the fields as flies
- All sterile flies are color marked
- No sexing strain; max. 10-15 generations from wild!



Monitoring the fly population in the field

- mark - recapture**
- sterile flies are marked
 - various colours



- mark - recapture**
- at least one set of traps / field
 - traps are emptied once per week
 - catches are processed immediately



Monitoring the fly population in the field

mark - recapture provides information on:

- Size of fertile population
- Ratio of fertile : sterile flies
- Age structure of the sterile fly population
- Recapture rate
- Effect of additional spray



Monitoring the fly population in the field

- The information enables to assess the risk on damage on each separate field and respond quickly

how many flies are required?
additional spraying?



Current situation

- In the last few years strong expansion to new areas within the Netherlands. For 2016 about 9.500 hectares
- Total area: 25.000 hectares
- Chemical alternative: seed treatment with Mundial
 - Easy and effective
 - Also effect on thrips
 - Seed treatment is no IPM
 - Does leave a residue in harvested onion



A unique way of working...

- Each field is treated separately
- Fully paid for by the growers; no subsidies!
- Price depending on size of the field and the crop
- Contracts per year
- Requires a good participation rate
 - To prevent contamination from non-participating fields
 - For efficiency in the logistics of fieldwork

Augmentative SIT?

FAO/IAEA Consultants' Group Meeting: Integration of the SIT with Biocontrol for Greenhouse and Other Confined Pest Insects.

IBMA
INTERNATIONAL BIOLOGICAL
MANUFACTURERS ASSOCIATION

Options for biological pest control;
experiences, regulatory framework, prospects

14–18 March 2016, Vienna, Austria.

Dr. Lieselot Van der Veken

bioBEST
SUSTAINABLE CROP MANAGEMENT

**Biological nematode control:
an agro-ecosystem approach**

LEUVEN
KATHOLIEKE UNIVERSITEIT
Laboratory of Tropical Crop Improvement

Bioversity International

- Potential of multipurpose intercrops for the management of pathogenic nematodes and beneficial arbuscular fungi and root nodulating rhizobacteria in banana-based cropping systems

6/8/2016

General objective

LEUVEN **Bioversity International**

Better understanding of the different biotic components in a mixed banana production system to develop more efficient integrated nematode management

**Biological nematode control:
an agro-ecosystem approach**

LEUVEN **Bioversity International**

6/8/2016

**Biological nematode control:
an agro-ecosystem approach**

LEUVEN **Bioversity International**

Conclusions

LEUVEN **Bioversity International**

Nematode susceptibility: cultivar specific.

AMF compatibility: majority of the IC's had intermediate compatibility

Rhizobial compatibility: very specific; few functional symbiosis were observed

Interactions:

- No effect of AMF or rhizobia in non/poor nematode hosts: marigold/sunn hemp
- AMF suppressed nematodes in intermediate/ good nematode hosts: sweet potato/ sorgho-Sudangrass
- Dual inoculation (AMF and rhizobia) suppressed nematodes in intermediate nematode host soybean
- Single or dual inoculation (AMF or/and rhizobia) suppressed nematodes in intermediate nematode host common bean

Common bean (and sorgho-Sudangrass) as potential intercrops

IC_{AMF} did not result in plant growth promotion, did not provide significant extra nematode suppression in mixed systems



Biobest Group

- Worldwide authority in pollination and biological pest control for protected crops since 1987
- Leading Brand
- N° 2 in a fast expanding market:
 - Geographically
 - Applications
 - Products
- 8 production units
- +350 employees
- Broad, reliable product offering:
 - Biological Pollination
 - Biological Control
 - Monitoring & Scouting
 - Accessories
 - Greenlab



Our Network

Subsidiaries
Distributors

What is IBMA?

- International Biocontrol Manufacturers Association
- Established in 1995, this year 20th anniversary
- Over 230 members
- Global (European focused) Association
- Strong growth from 10 original founding members
- Diverse membership
- SME's to multinationals
- Organic and biocontrol only to IPM and conventional
- Principally involved in agriculture and horticulture



Mission statement

- Raise awareness, both among decision makers and consumers, concerning the benefits of the biocontrol products.
- Ensure Biocontrol is at the forefront thinking. It is not just something that it is nice to do but a priority. (SUD)



4 professional groups according to product categories

 Microbials	 Macrobials	 Semochemicals	 Natural and Biochemical Products
Viruses Bacteria Fungi	Predatory mites Insects Nematodes	Pheromones Plant volatiles	Plant extracts Essential products Basic chemical substances




IBCAs: Scope

- Professional group head of the Invertebrate Biological Control Agents (IBCA's)

include insects, entomopathogenic nematodes and predatory mites



IBMA INTERNATIONAL BIOCONTROL MANUFACTURERS ASSOCIATION **PG IBCA organisation**

Steering Group:

- Chair: Lieselot Van der Veken, Biobest
- Vice Chair: Phil Walker, BCP
- Johannette Klapwijk, Koppert
- Enric Vila, Agrobio
- Samuel Critchley, Syngenta BL, Invivo
- Patrick Frettinger, BASF

IBMA INTERNATIONAL BIOCONTROL MANUFACTURERS ASSOCIATION **Objectives**

- **Main group topics:**
 - Promote IBCA's as sustainable solutions for crop protection in a wide range of crop
 - Promote reasonable and harmonized regulation of IBCA's
 - Provide input to the EC on regulations relevant to IBCA's, such as Invasive Alien Species, Access & Benefit Sharing
 - Address scientific issues together with experts to anticipate future regulation
 - Support national groups on IBCA regulation and other issues

IBMA INTERNATIONAL BIOCONTROL MANUFACTURERS ASSOCIATION **Position papers**

Position Paper on Regulation of Invertebrate Biological Control Agents

Invertebrate Biological Control Agents (IBCA's) are key elements in IPM systems in agricultural ecosystems, and especially greenhouses. They offer a safe alternative for the use of pesticides, and are effective also in cases where chemical and control tools become of less use. In greenhouses, the use of IBCA's is often seen as a natural local control element and not always seen as a control element and the introduction of exotic species may be required. Although there are hardly any examples of IBCA's causing problems for the environment, we have the obligation to assess the risks of releasing a species in a new environment prior to its release.

Current guidelines on IBCA regulation for the most and number of IBCA's, national requirements on safety of introduction and for exotic organisms, which involve the following requirements for the registration of IBCA's:

1. The risk to studies already conducted by IBCA, EPPO and REBECA on an existing list of the release of IBCA's (see also IBCA, REBECA, the experts, van Lenteren, 2002 and Klapwijk and Koppert, 2002).
2. A Risk Assessment should be primarily based on existing scientific knowledge, taking existing risk assessment tools, or related studies into account. Studies should be conducted in a greenhouse and submitted to competent national authorities.
3. The regulatory agency (national or EU) will use an integral system to bring the guidelines for IBCA's registration for each a decision.
4. In case that not all assessment criteria are covered by national data, additional requirements need to be introduced by the applicant in accordance with national authorities.
5. The Assessment body should also be based on the principles described in EPPO standard 22: Exotic control. For the Assessment should be taken into account in consultation with national authorities.
6. Risk assessments on non-target organisms should not focus on species commonly present in farms.
7. Specialist knowledge should be included in the same regulation as for pesticides and other organisms should not exceed those described in EPPO standard 22.
8. Competent national authorities should consult the regulatory body for further Risk Assessment being based on the dossier and in consultation with the applicant.
9. Higher standards of safety, quality, resistance, safety, etc. should be developed within IBCA's.

IBMA INTERNATIONAL BIOCONTROL MANUFACTURERS ASSOCIATION **IBCA's for key greenhouse pests EU**

biobest SUSTAINABLE CROP MANAGEMENT

IBCA's for key greenhouse pests

Predatory mites most important IBCA's

Species	Relative Importance
<i>Amblyseius swirskii</i>	65%
<i>Phytoseiulus persimilis</i>	~15%
<i>Neoseiulus californicus</i>	~10%
<i>Macrolophus pygmaeus</i>	~5%
<i>Encarsia formosa</i>	~5%
<i>Orius laevigatus</i>	~5%
<i>Nesidiocoris tenuis</i>	~5%
<i>Neoseiulus cucumeris</i>	~5%
<i>Eretmocerus eremicus</i>	~5%
<i>Aphidius colemani</i>	~5%

Top-10 of biocontrol agents used in greenhouses (turnover) (J. Klapwijk)

IBCA Regulation history:

- **1996** FAO ISPM 3 (IPPC)
- **1997** EPPO / CABI on Safety and Efficacy of Biological Control in EU: endorsement ISPM 3
- **1999** EPPO Guidelines for the first import of exotic BCAs for research under contained conditions
- **2000** EPPO Guidelines for import and release of exotic BCAs
- **2002** EPPO positive list with IBCAs widely used in the EPPO region
- **1998-2002** ERBIC; detailed criteria for RA and IBCA ranking (safety)
- **2003** OECD Guidance for information requirements for IBCAs
- **2003** IOBC/WPRS Commission for the Harmonisation of Regulation of IBCA's
- **2005** FAO: revised version of ISPM 3
- **2006** Bigler *et al.* 2006: book as framework for ERA of IBCAs
- **2006-2008**: REBECA (EU Policy Support Action)

(Ehlers, 2011)

REBECA project (EU 2006-2008)

- Need for balanced and appropriate EU regulatory systems for import and release of BCA's
- IBCA's : EPPO guidelines
 - Human health risk: usually limited
 - Environmental risks of exotic species (CBD)

How to evaluate IBCAs environmental risk?

Identify risks of introducing exotic natural enemy

- **Establishment** and/or **dispersal** in non-target habitat
- Non-target **host range**
- (In) direct effects on **non-target organisms**

Determine likelihood and magnitude of each of the risks
Quantify risk and apply cost-benefit analysis (also for other control methods!!!)

(Van Lenteren, 2006)

Stepwise risk assessment : from 5 to 1 scheme

- Clearly good or bad species are discovered early in evaluation (saves money and time)
- Only doubtful species go through whole evaluation
- Scheme can be used for quick scan or comprehensive evaluation
 - We tested 150 commercially available species with this scheme

Van Lenteren, 2006

Native natural enemy: all natives (34 spp.): safe

Van Lenteren, 2006

Native natural enemy all natives: safe

Exotic natural enemy for greenhouse use

If establishment impossible, usually safe

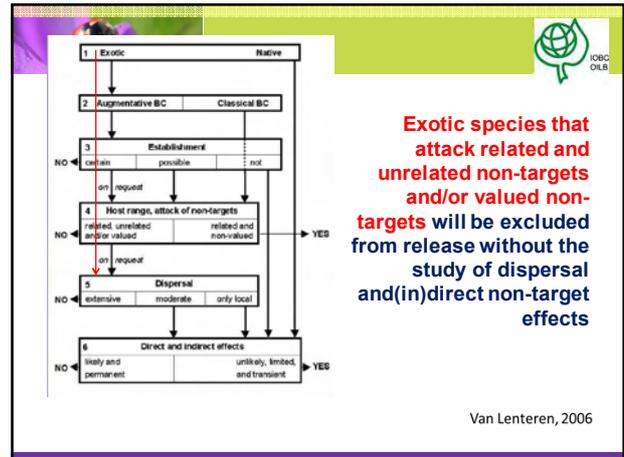
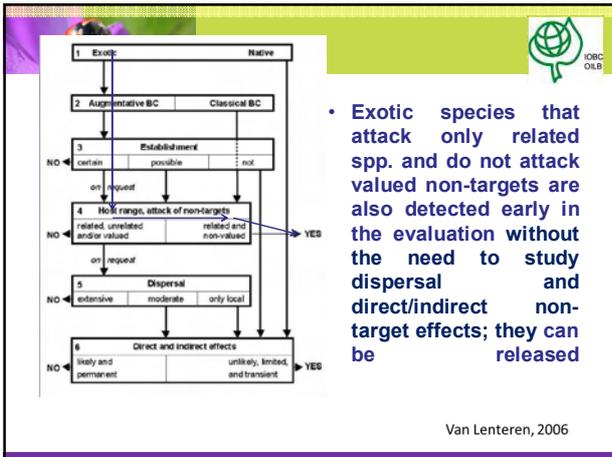
If establishment possible more work!

SIT

Van Lenteren, 2006

Exotic species for augmentative biological control that are likely to establish are detected very early in the evaluation process, and will be excluded from release without further studies

Van Lenteren, 2006



Conclusions application hierarchical screening

- All native species (34) considered safe for release
- Compared to earlier risk analyses: prevent unnecessary studies, quicker, cheaper, simpler

EPPO PM6(3) "positive list"

- http://archives.eppo.int/EPPOStandards/biocontrol_web/bio_list.htm

European and Mediterranean Plant Protection Organization
 Organisation Européenne et Méditerranéenne pour la Protection des Plantes

EPPO Standards on Safe Use of Biological Control - PM 6/3 - Version of October 2014

List of biological control agents widely used in the EPPO region

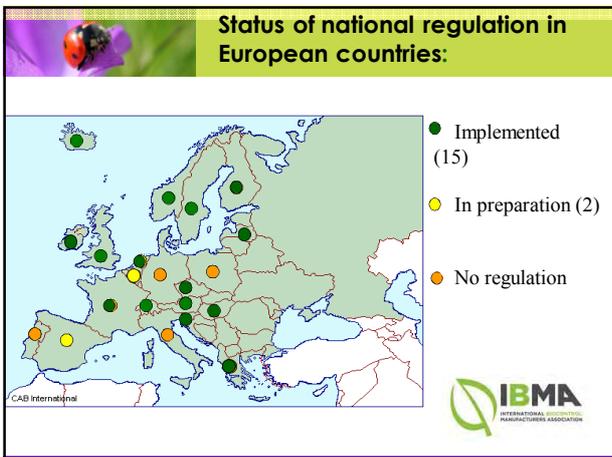
Specific scope: This document gives a list of biological agents widely used in the EPPO region, to facilitate decision on the import and release of biological control agents within EPPO countries.

Specific approval and assessment: First approved in September 2005. Revisions of the list are not subject to approval by EPPO Council, but are decided by the Panel on Safe Use of Biological Control.

Introduction and regulatory text:
 - Appendix 1: Commercially used biological agents
 - Appendix 2: Commercially introduced classical biological control agents
 - Appendix 3: List of biological control agents formerly recommended by EPPO

Download appendix 1, 2 & 3

EPPO Standards PM 6/1 and PM 6/2 provide guidelines to national authorities in the EPPO region on the introduction and release of non-indigenous biological control agents, so as to identify and avoid hazards for agricultural and natural biodiversity. These standards are intended to be used in addition to future introductions and use assessments for pest



International Regulation

- ISPM 3 (ISPM 2 PRA)
- NAPPO region: NAPPO application: US, CAN and MEX (exotic species disregarded)
- Rest of the world : country specific

Regulatory framework

ISPM 3: GUIDELINES FOR THE EXPORT, SHIPMENT, IMPORT AND RELEASE OF BIOLOGICAL CONTROL AGENTS AND OTHER BENEFICIAL ORGANISMS (2005)

SCOPE

This standard provides guidelines for risk management related to the export, shipment, import and release of biological control agents and other beneficial organisms. It lists the related responsibilities of contracting parties to the IPPC ('contracting parties'), National Plant Protection Organizations (NPPOs) or other responsible authorities, importers and exporters (as described in the standard). The standard addresses biological control agents capable of self-replication (including parasitoids, predators, parasites, nematodes, phytophagous organisms, and pathogens such as fungi, bacteria and viruses), as well as *sterile insects** and other beneficial organisms (such as mycorrhizae and pollinators), and includes those packaged or formulated as commercial products. Provisions are also included for import for research in quarantine facilities of non-indigenous biological control agents and other beneficial organisms.

The scope of this standard **does not include living modified organisms**, issues related to registration of biopesticides, or microbial agents intended for vertebrate pest control.

- * an insect that, as a result of an appropriate treatment, is unable to produce viable offspring

6/8/2016

Prospects

- Harmonized IBCA regulation within an ecological zone context (relevant a/biotic parameters limiting species distribution)



- Use EPPO list as a positive list with safe IBCA's
- Risk categories: ranking according to risk:
 - Develop tools based on these categories: the safer the category, the lesser assessment required
 - For specialist parasitoids less data required as for generalist predators



- Expected vs perceived risk
- Quick scan 150 species: 80 approved directly, 15 after assessment

Regulatory framework

- Depending on SIT technique

<p>Irradiation</p> <ul style="list-style-type: none"> ISPM 3 (ISPM 2 PRA) No establishment if QC guarantees sterility 	<p>Molecular</p> <ul style="list-style-type: none"> ISPM 11 (GMO) No establishment if QC guarantees sterility Acceptance in EU?
--	---

6/8/2016

Important crop pests: potential SIT?

Technical feasibility for SIT?

- sexual reproduction
- mass rearing
- adequate sterilisation by irradiation
- good fitness after irradiation
- no remating after mating sterile male
- island population criteria met in confined conditions

Important crop pests: potential SIT?

Key greenhouse pests in EU-N Am

Pest scientific name	Common name pest	Taxonomical group	Crop
<i>Aculops</i>	Tomato russet mite		Tomato
<i>Conopsea</i>			Tomato
<i>Drosophila suzukii</i>		Diptera	Soft Fruits
<i>Scirtothrips mangiferae</i> (blueberry - leaf thrips)		Thysanoptera	Soft Fruits
<i>Echinothrips</i>			Cut flowers
Mealybugs			Tomato, sweet pepper, ...
<i>Bemisia tabaci</i>		Hemiptera - Aleyrodidae	Several
<i>Trioletoides vaporariorum</i>		Hemiptera - Aleyrodidae	Several
Leafhoppers			Sweet peppers
<i>Frankliniella occidentalis</i>		Thysanoptera	Soft fruits, Sweet peppers, cut flowers, ...
<i>Necoris</i> sp.		Heteroptera	
<i>Lycus</i> spp.		Heteroptera	cucumber

Important crop pests: potential SIT?

- Currently absent in EU, big pests in other continents eg L-Am, Asia



Tomato psyllid (*Bactericera cockerelli*)



Pepper weevil (*Anthonomus eugenii*)



Eggplant stem borer (*Neoleucinodes elegantalis*)



Citrus psyllid/ huanglongbing (*Diaphorina citri*)

THANK YOU FOR YOUR ATTENTION



Occurrence and Control of Insect Pests on Greenhouse Vegetables in China

Shaoli Wang
March 14, 2016

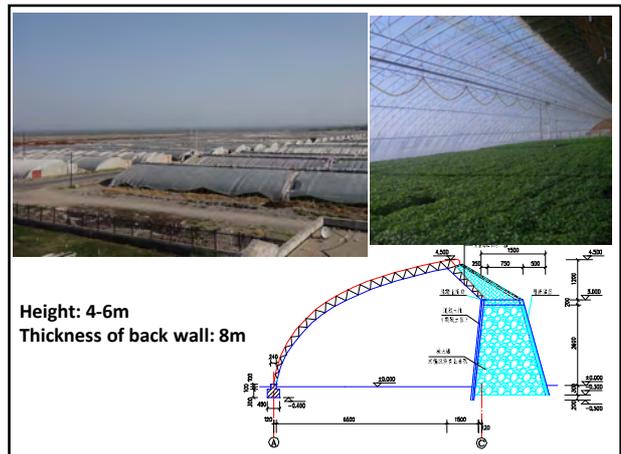
Outline



- Greenhouse in China
- Vegetable Ecosystem in greenhouse in China
- Main insects and their control in greenhouse in China
- Integration of SIT and biological method in China

Greenhouse in China

- Modernized greenhouse (Left)
- Solar greenhouse—widely used(Middle) 8-30°C
- Plastic shed—widely used(Right)



Importance of developing the protected cultivation in China

- Meeting the requirement of few kinds of vegetables in Northern China, even though this still needs much improvement
- achieve the vegetable supply throughout the year
- Some kinds of fruit trees and flowers
- The protected cultivation reaches 4 million hm² in China.

Vegetables in the greenhouse in China



Vegetable ecosystem in greenhouse in China

1. The ecosystem is unstable due to the short-term growing periods and the crop changes frequently.
2. The temperature changes drastically in the greenhouse ranging from 8 degree to 30 degree, especially in the early spring and late autumn.
3. The humidity in the greenhouse is very high, resulting in the plant disease.
4. The control of natural enemy on the greenhouse insect is relatively weak due to the unstable ecosystem, environment and the insecticide spray.

Characteristics of vegetable insect in greenhouse in China

(1) The occurrence of the insect pests becomes more serious

- ❑ Cultivation pattern is complicated. There are solar greenhouse, plastic shed and sunshade screen et al. making the complexity of the insect pests;
- ❑ The expansion of the greenhouse is helpful for the over-wintering or over-summering of some insect pests.
- ❑ The expansion of greenhouse and intensive cultivation make the greenhouse inadequate for the insects.



(2) Secondary pests become the main pests

- ❑ Stripped flea beetle is the most important insect on vegetables in Southern China and it becomes more and more serious in Northern China currently.
- ❑ The root maggot becomes a key factor impressing the production of onion and garlic in northern China.



(3) Invasive insect pests become more serious

- ❑ Leaf miners of *Liriomyza sativae* and *Liriomyza huidobrensis* were found in Hainan, Guangdong in 1994
- ❑ Sweetpotato whitefly, *Bemisia tabaci* B biotype was found in Beijing, Hebei, Guangdong at the end of last century.
- ❑ West flower thrips, *Frankliniella occidentalis*, was found in Beijing in 2003
- ❑ *Bemisia tabaci* Q biotype was found in Beijing in 2003
- ❑ American serpentine leaf miner, *Liriomyza trifolii* appeared in South China in 2006



(4) Controlling difficulties for the small insects and intermittent outbreak of some pests

- ❑ Small insects including whiteflies, leaf miners, thrips, spider mites and the aphids occur in the protected cultivation throughout the year and the population increases rapidly.
- ❑ Beet armyworm, *Spodoptera exigua* is an important insect pest with the characteristics of intermittent outbreak in some areas.



(5) Insecticide resistance of some insects is prominent

- ❑ Insects in greenhouse undergoes higher selection pressure against insecticides than those on other crops due to the frequent insecticide spray.
- ❑ Few insecticides for the vegetable insects can be used.
- ❑ Diamondback moth, beet armyworm, whitefly, west flower thrips and the two spotted spider mite are the most prominent insects in greenhouse.



Main insects in greenhouse in China

- Whitefly**
Greenhouse whitefly (*Trialeurodes vaporariorum*)
Sweetpotato whitefly (*Bemisia tabaci*)
- Thrips**
Onion thrips (*Thrips tabaci*); Thrips palmi
Western flower thrips (*Frankliniella occidentalis*)
- Aphid**
melon aphid (*Aphis gossypii*); Green peach aphid (*Myzus persicae*)
- Leafminer**
Pea leafminer (*Liriomyza huidobrensis*); Vegetable leafminer (*Liriomyza sativae*)
- Mites**
Broad mite (*Polyphagotarsonemus latus*); Tetranychus spider mite (*T. urticae* and *T. truncatus*)
- Melon fly**
melon fruit fly (*Bactrocera cucurbitae*); Oriental Fruit Fly (*Bactrocera dorsalis*)



Other important insects



Whiteflies

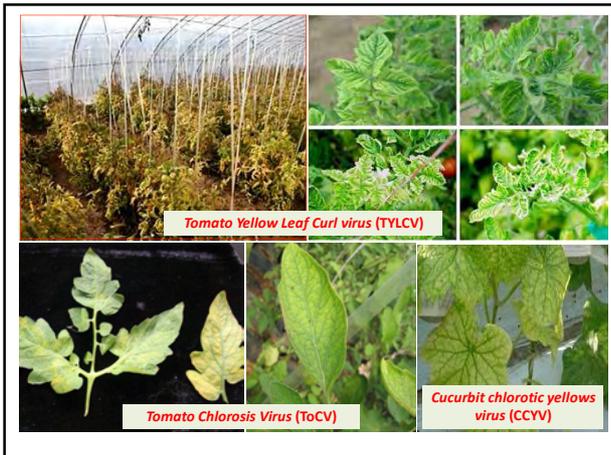
There are four life stages: egg, nymphal instars, pupal stage, and the adult.



Sweetpotato whitefly
Bemisia tabaci

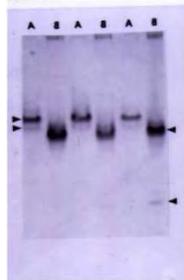
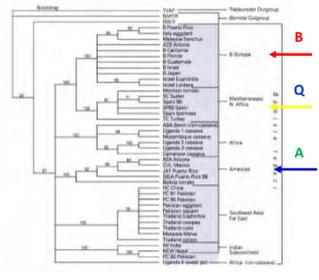


It can cause great damage to a broad range of plants (over 600) by direct feeding, excretion of honeydew.



Biotype (Cryptic species)

Costa & Brown (1990): A, B, Q et al. were identified using the pattern of esterase, mt COI genes and other methods.

B biotype

- ❑ Mid 1980s to 1990s: Major geographical expansion in B-biotype
- ❑ B-biotype become predominant due to wide host range, resistance to insecticides and/or other characteristics.
- ❑ B-biotype is generally resistant to pyrethroids, but resistance to newer insecticide groups still patchy.

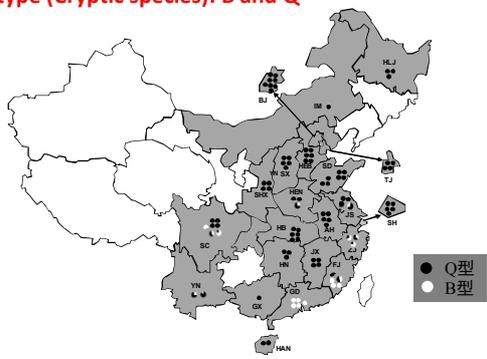
Q biotype

- ❑ Q-biotype was formerly restricted to Mediterranean, where resistance to a broad range of insecticides (neonicotinoids, IGRs, pyrethroids) is now widespread
- ❑ Q-biotype currently common over much of the Mediterranean, but not ubiquitous (B-biotypes still occur).
- ❑ Q-biotype (plus multiple resistance) are now being transported on ornamentals to other parts of the world (e.g. USA, northern Europe, Japan, and China)



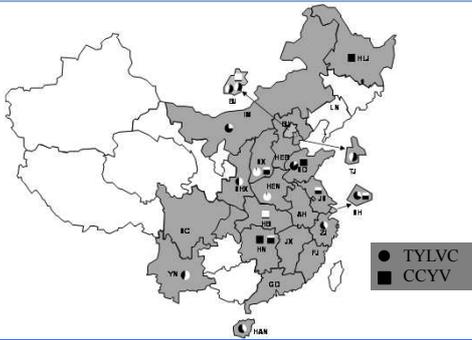
- ◆ From 2004 to 2007, B biotype predominated in most parts of China.
- ◆ Q biotype and B biotype coexist in mid-east parts of China. In some places, Q biotype replaced B biotype completely.

Biotype (Cryptic species): B and Q

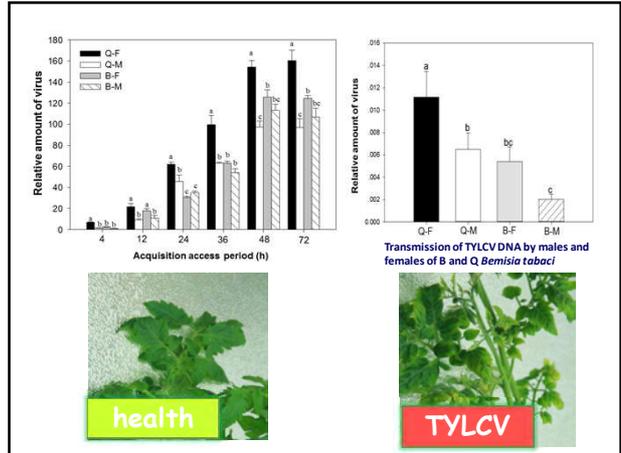


In 2014, 76 populations (Q), 10 populations (B), 7 populations (B+Q)

Rates of *B. tabaci* carrying TYLCV and CCYV



In 2014, 13 populations (of 22) collected from 11 locations were proved to have YLCV; 9 populations (of 21) from 15 locations carried CCYV.



Higher resistance of Q biotype of *B. tabaci* against different insecticides than B

Insecticides	Biotype	SE(SE)	LC ₅₀ (mg/L)	95% FL	Q/B
Abamectin	B	1.85(0.200)	0.0870	0.0630-0.120	
	Q	2.10(0.196)	0.0970	0.0740-0.127	1.12
Acetamiprid	B	3.47(0.358)	3.22	2.44-4.24	
	Q	1.49(0.157)	22.1	15.8-30.1	6.87
Thiamethoxam	B	1.06(0.112)	17.8	9.78-32.5	
	Q	0.602(0.0840)	54.9	19.2-157	3.08
Cyantraniliprole	B	1.58(0.111)	6.23	4.89-7.94	
	Q	1.12(0.145)	93.9	50.1-175	15.1
Chlorantraniliprole	B	1.13(0.245)	561	176-1788	
	Q	1.65(0.185)	4564	3071-6786	8.14
Buprofezin	B	1.98(0.148)	1476	1192-1827	
	Q	1.85(0.157)	5155	3639-7302	3.49
Pyriproxyfen	B	2.36(0.211)	1434	1125-1829	
	Q	1.58(0.183)	8810	5828-13319	6.14
Spirotetramat	B	1.51(0.209)	2450	1628-3687	
	Q	>16000	—	—	>6.53

Xie et al., 2014

Biological differences of *B. tabaci* B and Q biotypes

- Adaptability:** The adaptabilities, growth and development, of biotypes B and Q on the host plants are obviously different.
- Susceptibility to insecticides:** The *B. tabaci* Q biotype has higher tolerance or resistance to many insecticides than B, especially the neonicotinoid insecticides.
- Efficacy and characteristics of virus transmission:** The Q biotype has the stronger transmission efficacy than B for Tomato Yellow Leaf Curl Virus(TYLCV) and Tomato chlorosis virus (ToCV).



T. vaporariorum

Suppress the whitefly population

Non-host crops of *T. vaporariorum*, such as Chinese cabbage and celery

Cut off the host chain regionally

Conventional winter crops, such as cucumber and tomato

replace

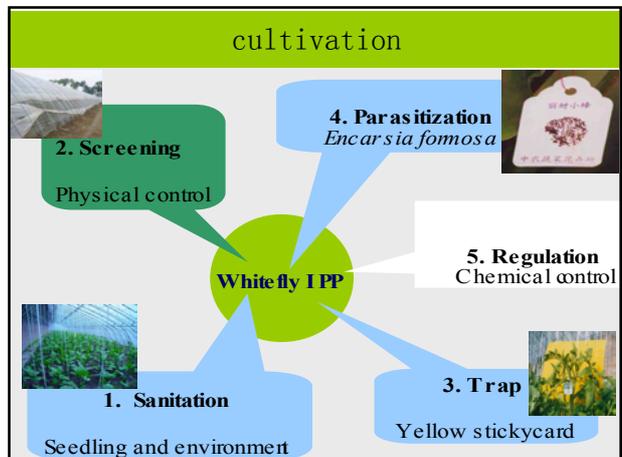
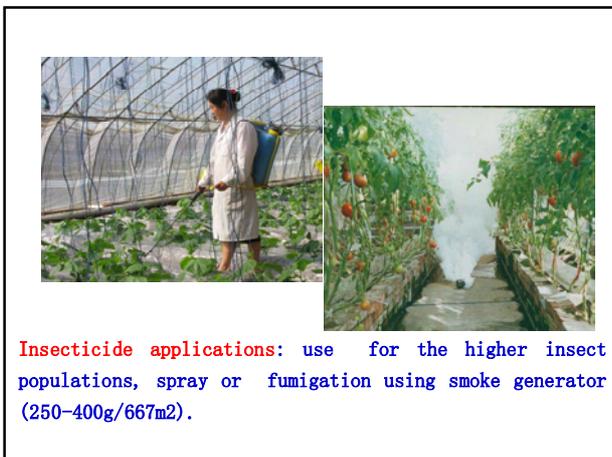
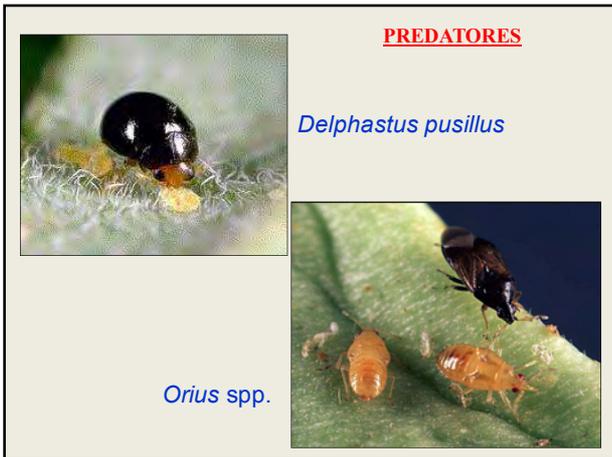
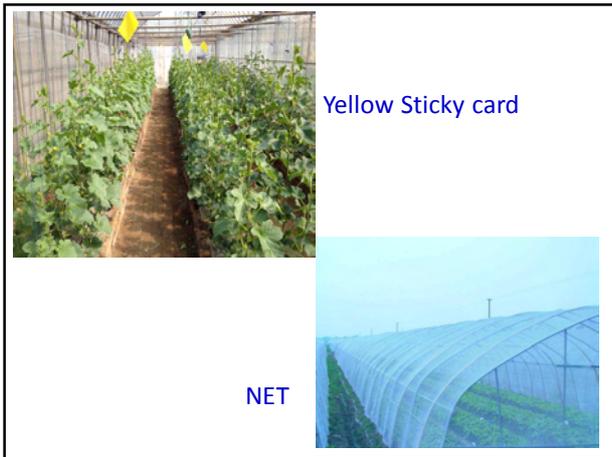


Insect-free greenhouse (insect-proof + sticky cards)



Different Vegetable Seedlings shouldn't bred together.

Healthy and clean seedlings: healthy seedlings needed to slow down the basic population of the insect pests



Thrips



Egg

First nymph

Second nymph



Pupa

Adult (female)

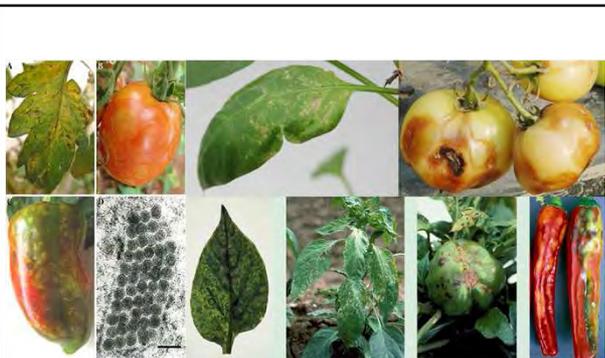
Adult (male)



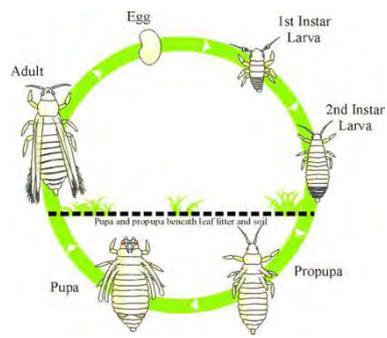
- Order Thysanoptera
- around 5,000 species have been described
- generally tiny, 1 mm long or less
- having a single mandible (jaw) used for rasping
- feed on plants and vector of some important virus



Western Flower thrip, *Frankliniella occidentalis* is an important invasive insect in China.



Tomato spotted wilt virus, TSWV is transmitted by this thrip and causes giant economical losses.



Adult female thrips insert eggs into plant tissues.

The late 2nd instars crawl down into the soil to pupate.

Life cycle of thrips



BIOLOGICAL CONTROL

PREDATORS: PIRAT BUGS. (*Orius spp.*)



PREDATORY MITES

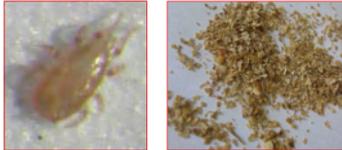


Amblyseius cucumeris

BIOLOGICAL CONTROL

Minute Flower Bug (*Orius tristicolor*), and two predatory mites, *Amblyseius cucumeris* and *Hypoaspis miles*. Minute pirate bugs are polyphagous and will also feed on aphids, mites, and small caterpillars. *Orius* are released at a rate of 2000 to 4000 per acre, while *N. cucumeris* are released at a rate of 10 to 50 mites per plant for each of 2 to 3 weeks.

These mites will also feed on spider mite eggs, pollen, and fungi. *Hypoaspis miles* are soil-inhabiting predators that feed on thrips prepupae and pupae in the soil. A commercially available parasite of greenhouse thrips is *Thripobius semileteus*.



CHEMICAL CONTROL



Root irrigation using imidacloprid or thiamethoxam (3000-4000x) after the seedling transplantation with 30ml/plant.

Spinetoram, abamectin, thiamethoxam, emamectin benzoate are the candidates using in the greenhouse currently.

Leaf miner



Liriomyza sativae



BIOLOGICAL CONTROL The parasites *Diglyphus* spp. is commercially available to control leafminers and may be useful in greenhouse situations, especially if greenhouses are screened to exclude adult leafminer movement into greenhouses.



www (Diglyphus sp.)

LARVA EATING THE MINER FLY MAGGOT

PUPAE

Aphid



Control methods for aphids

- 1) **Cultural control.** clean the weed and make the seedlings clean and healthy
- 2) **Seedlings with mesh.** White or silver grey 40-50 mesh are used to cover the seedling beds to minimize the aphids and its vectored virus disease.
- 3) **Preventing aphid with the plastic film.** Silver grey mesh is used to cover the door and window, ventilation opening, in case of the aphid flying into the greenhouse. Silver strip is hung in the field.
- 4) **Sticky card.** Yellow sticky cards are hung in the field with the upper margin similar with the plant.
- 5) **Chemical control.** Chemical control. ①Root irrigation; ②Partial control; ③Spray control; ④Smoking method

Spider mite

DAMAGE Mites suck cell contents from leaves, initially stippling leaves with a fine pale green mottling. As feeding continues, the stippling increases and leaves turn yellow with bronzed or brown areas; damaged leaves frequently fall.



CULTURAL CONTROL

Because spider mites feed on a large variety of plants, keep production areas free of weeds, which can serve hosts to the mites. Carefully inspect plants being brought into a new crop to ensure that they are free of mites.



BIOLOGICAL CONTROL

Many different species of predatory mites are available for control of the mites. *Phytoseiulus persimilis* is a commercially available predator of two spotted spider mite, and it has been used to control mite populations in greenhouses and field situations. It can reproduce faster than its prey, yet best results can be obtained when it is released into the crop before the spider mite populations have built up.



Lepidoptera moth

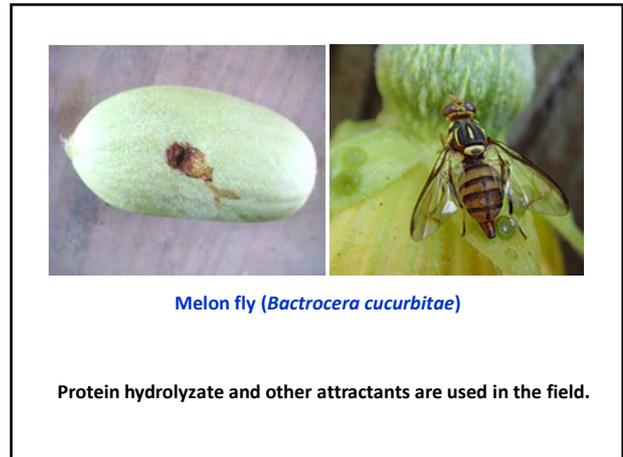
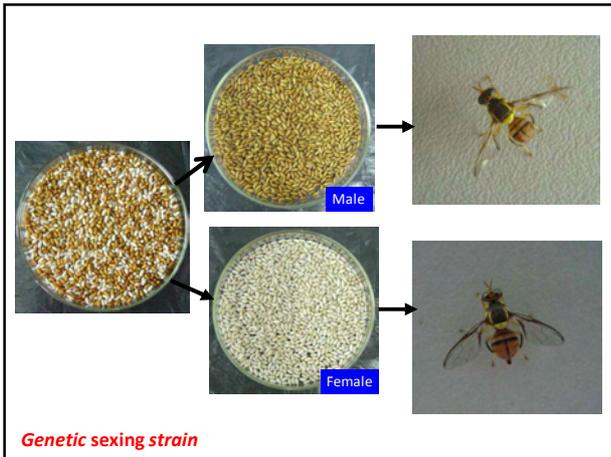


Potential of Integration of SIT and biological method in China



Jiahua Chen and Qinge Ji Fujian Agriculture and Forestry University

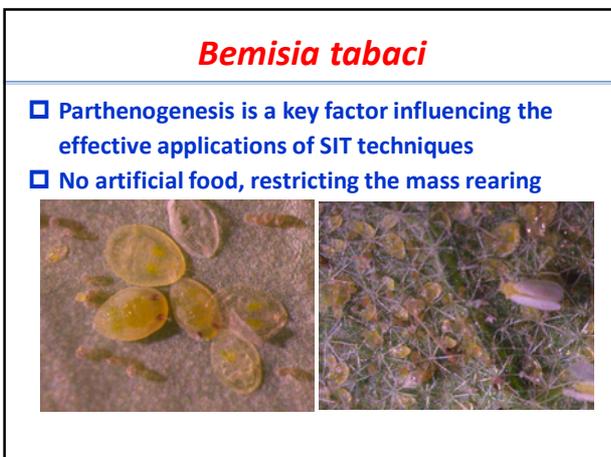




Leaf miner

It's possible to control through the integration of biological and SIT techniques, even though there are many steps to go.

- (1) bisexual reproduction
- (2) It's possible to realize the mass-rearing through artificial food



Constraints to the integration of biological control and SIT

- ❑ No SIT techniques have been developed for most other important insects in greenhouse up to now
- ❑ The mass-rearing of the parasitoids or predators are limited in China
- ❑ The environment in greenhouse is not helpful for the population establishment of the natural enemies
- ❑ The oriental fruit fly and the melon fly has the SIT techniques, but their parasitoids are still in the laboratory
- ❑ The control effects of integration of biological control and SIT technique needs to be evaluated.

Thank you for your attention !





Counterparts

- PPIS

National Contributors

- Bio-Bee Sde Eliyahu Ltd.

Liriomyza bryoniae

- Major species in Israel
- Highly polyphagous pest of vegetables and flower crops, widely distributed throughout Asia, Europe and Africa
- A worldwide pest, is not considered a quarantine pest in any country but Ireland, therefore SIT could be useful for many countries.

TC Objective

Develop / enhance biological control for leaf miners using SIT

Output 1:
Develop/improve mass rearing technologies for leaf miner flies

Activities:
Improve the modular system for mass-production of the fly at Bio-Bee

Improve current mass production technique of the leaf miner *Liriomyza bryoniae*

Develop novel mass rearing technique for leaf miners based on artificial diet

Results

- An upgraded mass rearing system for leafminers was achieved.

New vs. old production facility: Yield improvement, of over 30%, was achieved by the newly developed prototyped greenhouse for leaf miners mass rearing.



- Preliminary studies were carried out on artificial diets.
- However, an artificial diet for *Liriomyza* has yet to be achieved




Output 2:
Determine Irradiation dosage for the leaf miner flies

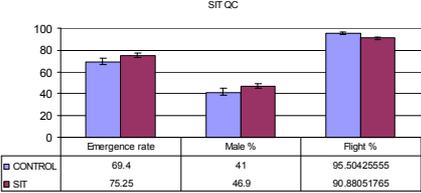
Activities:
Age of the pupae when irradiated
Handling (reduced-oxygen etc.)
Optimal dose for sterilization
Quality of sterilized vs. normal flies:

- Emergence and flight ability
- Longevity under stress
- Dispersal
- Mating performance (competitiveness)
- Survival in the field



Results

Sterilized leaf miners were tested for emergence rate, sterility and flight capacity according to the medfly QC protocols.

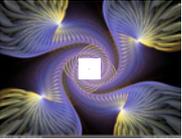


	Emergence rate	Male %	Flight %
CONTROL	69.4	41	95.50425555
SIT	75.25	46.9	90.88051765



Findings

- Optimum stage for irradiation: Late pupae (close to eclosion)
- Keep under hypoxia conditions
- Optimum dosage 170Gy
- Ensures efficient sterilization while maintaining a high quality of emerging adult leafminers




- **Output 3:**
- Emergence and release technologies
- Irradiation performed under hypoxia and pupae released in aired plastic containers



- **Output 4:**
- Perform pilot tests assessing the effect of releasing sterile leafminers in greenhouses

Activity: SIT pilot tests for SIT efficacy

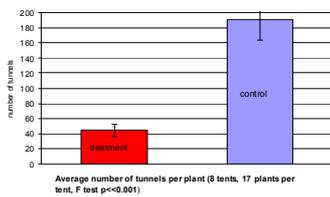
Pupae of *L. bryoniae* were dispersed on tomato plants in a greenhouse.

Preliminary test with a ratio 1:2 fertile:sterile flies was rejected (40% decrease).

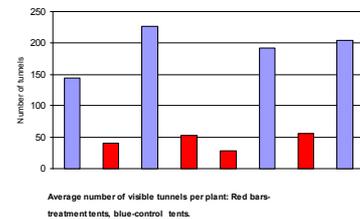
Test replicates received a ratio of 1:5 fertile:sterile flies.

Observations were carried out throughout the infestation stage and proved, early on, the efficiency of the sterile flies in reducing the amount of tunnels on the leaves.

Significant decrease of over 75% in infestation (based on tunnel count) was recorded.



12 days after introducing *L. bryoniae* to the tomato plants, the total numbers of visible tunnels were counted.



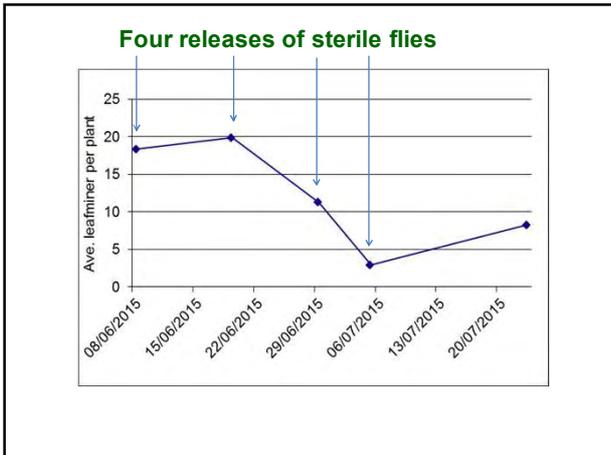
Activity :

Test the efficiency of a supposed synergism between the leaf miner's natural enemy, *Diglyphus isaea* and the sterile flies



Commercial tomato greenhouse (2000 m²) test

- Treated before, during and after with the biological control agent, *Diglyphus isaea*
- At ten day intervals 160,000 sterile *Liriomyza bryoniae* flies (at a ratio of 1:5 fertile/sterile) were released during a 5 week period
- An over 80% reduction in leafminer population proving that the method can work on a commercial scale.



Constraints identified from project



- **Artificial diet for *Liriomyza*** (conflict between natural enemy rearing and sterile fly production).
- **A technique to distinguish between leafminers' gender, while in larval or pupal stage.** (♀ leaf stings – nutrition and sterile egg laying)

Other Issues for consideration on the use of SIT in confined areas (1)

- **Production numbers** would have to be much higher than equivalent, if available, natural enemies.
- If a natural enemy is available will need to perform **equivalency tests** N/E vs. SIT and also synergy.

Other Issues for consideration on the use of SIT in confined areas (2)

- **Commercial viability** vs. traditionally (sprays) will also need to be taken into account.
- Perhaps better to concentrate on **pests that have no efficient natural enemies – BUT** then mass rearing knowledge is likely to be lacking.

