WORKING MATERIAL

Development of a protocol for the planning and implementation of a pilot trial using the sterile insect technique against codling moth in selected European target areas

Report and recommendations of a consultants group meeting organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, Austria, 13-17 February 2017

Reproduced by the IAEA

Vienna, Austria 2017

Executive Summary

The codling moth (CM), *Cydia pomonella* (L.) is a key pest of most pome fruit (apple, pear and quince) and some walnut orchards in the temperate regions of the world. There has been an increase in awareness of the negative consequences of the use of insecticides in agriculture. There has also been a withdrawal and banning of a number of essential CM control insecticides because of increasing environmental and human health concerns. Pome fruit growers are being faced with serious challenges to effectively manage CM in their orchards and to place their pome production in international markets.

The sterile insect technique (SIT) has proven to be an environmentally friendly, effective control tactic for CM. The approach entails the mass production and sterilization of CM and the sustained release of the quality reared insects in numbers large enough for them to outcompete the wild males mating with wild females.

A group of consultants was brought together to plan and develop a protocol to facilitate the effective implementation of pilot programmes that would rely on the release of sterile insects to manage CM in pome fruit and walnut orchards in selected target areas in Europe and the United States.

With this objective, the following document was written to provide a general pilot trial procedure and the country-specific recommendations included in the appendix. Procedures include guidelines on the collection of baseline data of wild codling moth populations in the selected trial locations. Each participating country proposed specific start times, treatments and plot sizes to create the most effective control tactics for their region. The consultant group decided upon pilot trial design and release strategies which are laid out for future trial participants in this report. Guidance on specific monitoring strategies is also included.

A number of other pilot trial parameters are considered in the document including permits for releases, sterile moth import, and regulatory issues. Moth production quality control is discussed. The report also includes a communication strategy and a list of possible project stakeholders who might be valuable partners when setting up the pilot trails.

Contents

1. Introduction

A group of experts in the area of sterile insect release in agriculture assembled in Vienna, Austria from February 13 to 17 2017 to develop a detailed action plan / protocol to implement pilot trailsusing the sterile insect technique (SIT) against codling moth. The consultants in attendance represented Austria, Canada, France, Germany, Greece, Italy, Netherlands, the United Kingdom, and the United States (list of participants - Appendix B).

After a day of presentations from collaborators, the group began discussing and working on developing trial protocols. This report was drafted by the group, each country contributing and creating their own country-specific guidelines (Appendix C).

2. Background

The codling moth (CM), *Cydia pomonella* (L.) is a key pest of most pome fruit (apple, pear and quince) and some walnut orchards in the temperate regions of the world. Infestation in neglected apple and pear orchards can reach 60-80%. Keeping fruit-growing areas free of this moth or at a very low prevalence will allow the marketing of high-quality fruit with very low levels of insecticide residues. Some 84.7 million metric tonnes of pome fruits were produced from 5.2 million ha worldwide in 2014. The international trade in pome fruits was more than 6.4 million tonnes, valued at US $6,120 million (FAO STAT 2013).

In the past, control of the CM has relied mainly on the intensive use of organophosphate (OP) and other broad-spectrum insecticides at a considerable cost: in the southern USA, direct costs of conventional insecticide sprays plus residual fruit damage are estimated at about 3.5% of crop value, but indirect costs due to loss of natural enemies and pollinators, insecticide residues and environmental contamination are estimated to be up to 6 times this level. Although new developments such as the use of temperature-dependent models and more efficient pheromone traps has decreased the number of insecticide spraying cycles, the indiscriminate use of insecticides has led to the development of resistance and cross-resistance to most traditionally used organophosphore and pyrethroid insecticides, and even in some areas to the insect growth regulators (IGRs). The application of these insecticides has in addition resulted in the disruption of the natural control of the secondary pest complex.

In view of increased awareness on the negative consequences of insecticide use and the withdrawal and banning of a number of essential insecticides due to increasing environmental and human health concerns, the growers have been facing serious challenges to effectively manage CM in their orchards and to place their pome production in international markets. This has given rise to a series of alternative control tactics such as the use of selective, synthetic IGRs, mating disruption used alone or integrated with cultural practices (post-harvest fruit removal, tree banding to catch over-wintering larvae), "attract and kill", and biological control agents such as the use of the CM granulosis virus (CpGV). Like with all control tactics, these have advantages (more friendly to the environment, reduced reliance on insecticides) and some limitations (more effective under low population pressure).

In addition to the above, the sterile insect technique (SIT) has proved to be an environmentally friendly, effective control tactic that has been used with great success for a number of dipteran and lepidopteran insect pests, including the codling moth. The approach entails the production of the target insect in large numbers, the sterilization of the male sex, and the sustained release of the reared insects in numbers large enough for them to outcompete the wild males for mating with wild females. The goal is to greatly reduce or even eliminate fertile matings and thus offspring. The initial development of the SIT for CM control occurred almost simultaneously in Canada and Europe, but only in Canada was there a practical follow-up to use sterile males to control the CM. A CM SIT pilot programme was conducted from 1976 (320 ha) to 1978 (526 ha) in apple and pear orchards in the Similkameen valley of British Columbia. After the suppression of the native CM population with chemicals, one million sterile moths were released every week which exceeded the desired sterile to wild moth ratio of 40 to 1. Eradication was achieved in some localised areas and fruit damage was below the economic threshold (0.5%) in 98.9% 96.9 % and 100% of the treated orchards in 1976 (n=86), 1977 (n=193) and 1978 (n=157), respectively (Proverbs et al. 1982).

This successful trial culminated in the first operational CM SIT programme in the world that covered 8,000 ha of apple and pear orchards located in the Okanagan region of British Columbia, Canada (1992) (Dyck et al. 1993).

The Okanagan Kootenay Sterile Insect Release (OKSIR) programme started releasing sterile insects in 1994 with the initial goal to eradicate CM from the Okanagan and surrounding areas. Codling moth suppression was a success, as evidenced by significant reduction in wild moth trap catches, significant reduction of orchards reporting CM damage, and a significant reduction in the sales of OP pesticides used to control the CM. Despite this success, the cost of treating the tens of thousands of feral and unmanaged pome fruit trees in urban areas was too great, and the programme's strategy transitioned from eradication to suppression in 1998.

The OKSIR programme is the world's longest-running and most successful area-wide integrated pest management programme for the suppression of codling moth. The progamme currently services 3550 ha of apples and pears, situated amongst a diversity of other agricultural uses. Sterile insect release, population monitoring and enforcement are the main services offered by OKSIR. Because of the large urban/rural interface and the relatively small average orchard size (2 ha), enforcement and management through cultural practices in urban areas have also contributed to the programme's success. The programme has achieved less than 0.2% damage in greater than 90% of all commercial pome fruit acreage and reduced insecticide use to control CM by over 96% in the valley (from 50,000 kg of chemicals in 1991 to < 3000 kg in 2015). A recent benefit-cost analysis showed the economic efficiency of the programme, i.e. a benefit to the producers from insecticide cost savings, monitoring cost savings and reduction in codling moth injury amounting to CAN $ 395/acre (versus CAN $ 377/acre for mating disruption) (CAN $ 395 = US $ 307). The economic benefits per acre of orchard were much higher using the OKSIR strategy as compared to using conventional insecticides: the overall benefit-cost ratio of the SIT program was 1.19 for the producer and 2.51 in total (Cartier 2014). The programme continues to strive to increase efficiency and provide better services to the growers and community.

With eradication of CM from the Okanagan and surrounding areas no longer an objective of the OKSIR programme, the moth production capacity of its rearing facility is not being fully utilized. The opportunity for OKSIR to increase moth production and sell those surplus moths to other areas has many potential benefits. This would not only increase the efficiency of the OKSIR programme by offsetting its costs, but benefit pome fruit growers around the world with additional management options. Despite being an effective, economic and environmental friendly pest management strategy, SIT is often not adopted because the initial capital investment required is significant and typically requires unanimous, area-wide adoption (i.e. total pest population control). However, with the initial capital investment in place and technological aspects of the programme already developed by OKSIR, there is an opportunity to take advantage of this infrastructure and technical expertise. For these reasons OKSIR is working to offer sterile moths to growers outside of Canada.

3. Objective

The objective of this document is to provide a protocol to facilitate the effective implementation of pilot programmes that would rely on the release of sterile insects to manage CM in pome fruit and walnut orchards in select European countries and the United States.

4. Protocol Procedures

4.1. Base line data collection

Before any programme begins, it is necessary to collect baseline data about the local wild populations. The basic concept of the SIT is to release a population of sterile insects that exceeds the local wild populations by a large ratio. It has been suggested that this 'male over-flooding ratio' should be as high as 40:1 in the case of CM. In order to achieve this initial ratio, it is necessary to measure or estimate the abundance of the local wild CM population. Adams et al. (2016) describes a method for measuring the population size from catch data in a single trap (See Appendix A). Catch numbers of moths, fruit injury counts, or tree banding from the previous year can be used to estimate wild population densities and to determine the number of moths needed to achieve a 40:1 ratio. In orchards with high wild populations (fruit injury above 1 to 2% or more) from the previous season, higher release ratios may be required unless measures can be taken to reduce the population before SIT releases begin. It is best to avoid conducting trials in sites where nearby orchards or wild hosts could result in large immigration of mated gravid females.

4.2. Handling of sterile moths

Mass-reared CM irradiated with 150 Gy will be imported from the OKSIR facility in Osoyoos, British Columbia, Canada. Moths will be shipped overnight in insulated containers and released in orchards as early as possible after they are received. Optimum release time will be selected by individual researchers based on regional temperature and logistical conditions. Various tests of the competitiveness of mass-reared sterilized codling moths after chilling and air transportation have revealed limited reduction in response to pheromone, flight ability, mating ability, fecundity, fertility or longevity (Bloem et al. 1999, Blomefield et al. 2011, Carpenter et al. 2013).

4.3. Selection of control tactics

Initial field trials will focus on testing the effectiveness of mass-release of sterile moths for managing CM in commercial orchards. Fruit growers currently rely on insecticides, CpGV, pheromone-based mating disruption or a combination of these tactics for CM control. Thus, we propose to evaluate the efficacy of the following sterile release programmes:

1) release of sterile moths alone,
2) release of sterile moths plus judicious use of insecticides,
3) pheromone-based mating disruption overlaid with the release of sterile moths, and
4) granulovirus overlaid with the release of sterile moths.

Trials should include the appropriate control for each of the treatments: 1) no treatment, 2) insecticides only, 3) mating disruption alone or 4) granulovirus alone, i.e. no sterile male releases. If growers´ spray programmes impact trial blocks, it has to be ensured that control and experimental plots are treated the same. Table 1 lists the proposed treatments by participants in the project. It is recognized that the release of sterile moths and mating disruption seem to be incompatible technologies, in that one prevents mating while the other requires mating to achieve effectiveness. However, Judd and Gardiner (2005) found that combining the two technologies provided significantly better control of CM than either technology used alone. Matings often occur in orchards treated with pheromone and a proportion of those matings will likely be with sterile males. Thus, the two approaches are complementary rather than antagonistic.

The trials are estimated to last for three years.

Table 1: Proposed start time, treatments and plot size by participants in the project.

Country	Year 1	Crop	Treated plot size	SIT treatment	Control*
Austria		Apple		SIT + GV	GV
UK		Apple, Pear	4 ha	SIT + GV	GV
France	2018	Walnut	4 ha	SIT	GV
		Walnut	4 ha	SIT	Conv
Germany	2018	Apple	4-8 ha	SIT + GV	GV
Greece	2017/18	Apple, quince	4-6 ha	SIT + Conv.	Conv.
				SIT + MD	Conv.

				SIT	-0-
Italy North	2017	Apple	5 ha	SIT + MD	MD
Italy Rome	2017	apple	2 ha	SIT	-0-
Netherlands	2018	Apple, pear	5-6 ha	SIT + MD	MD
Michigan, USA	2017	Apple	4-8 ha	SIT + MD /Conv.	MD/Conv.

*GV = Granulovirus, MD = Pheromone based mating disruption, Conv. = Conventional CM control

4.4. Experimental design

All research will be conducted in commercial orchards in regions where CM is problematic. Field plots will consist of large commercial orchards subdivided into smaller plots or pairs of smaller orchards that are in close proximity. We propose a minimum plot size of 4 ha if possible. All sites should include a SIT release and a control where SIT is not being released. The experimental design should allow for proper statistical analysis of the results. There will be a minimum of 120-meter buffers between the treatment blocks. Smaller buffers may be used in pheromone-treated plots.

4.5. Release strategies

A total of 1800 moths/hectare (900 males) will be released weekly over the course of flight of each CM generation. Previous research on CM movement in apple orchards revealed that released males spread quickly over a fairly large area, dispersing more than half a hectare in a single evening (Adams et al. 2016). Thus, moths will be released in batches of 200 from each of 9 locations/hectare. Release sites will be interspaced by 33 meters within each plot (Figure 1). Moths will be released either by gently tossing them into the tree canopy or by placing them in paper bags attached to the trunk of the tree.

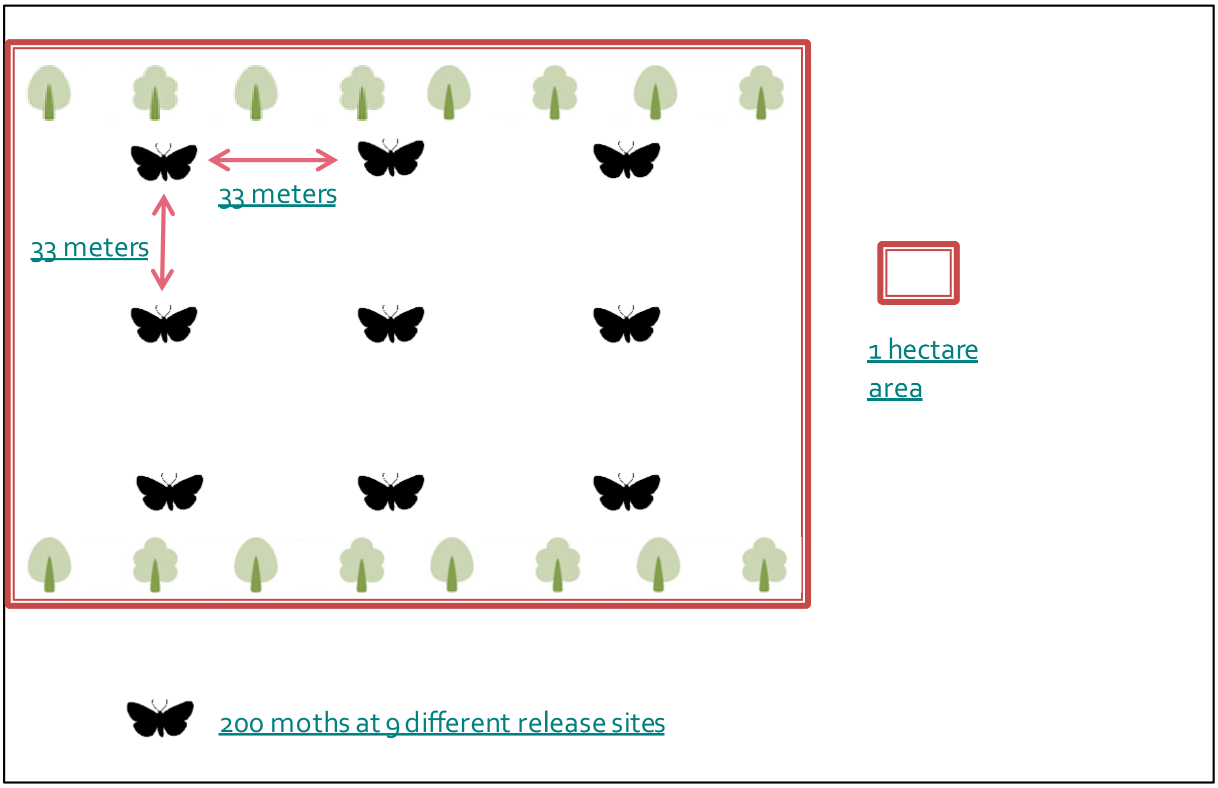


Figure 1. Example of a release plot where 1800 sterile codling moth are released per hectare/per week

4.6. **Monitoring strategies (moths/fruit damage)**

Captures of wild males in pheromone-baited traps, fruit injury counts after the flight of each generation and, optionally, captures of sterile moths marked with a fluorescent dye in traps will be used to assess treatment effects, i.e. the extent to which adult densities and larval infestation are reduced by treatments, and the extent to which male orientation is inhibited. Wild and sterile males can be readily distinguished as the sterile individuals are internally marked with a red dye that is incorporated into their diet. Six to nine Delta-style sticky traps baited with standard Trécé L2 pheromone lures will be deployed in the upper canopy in the central area of each plot, at least 30 m apart and away from the borders. Traps will be checked at least weekly throughout CM flights and cleared of moths before the next release. If catches are high, traps will be checked more frequently to avoid interference. Fruit injury will be assessed by inspecting 1000 fruits per hectare in the central area (20 from each of 50 trees) for the presence of CM infestation (stings or frass). Where oriental fruit moth (OFM) co-occurs, damaged fruit will be cut open and any larvae present will be confirmed as CM by the absence of anal combs (Figure 2).


Figure 2. (left) Posterior ventral view of anal combs of the larva of Oriental fruit moth larva, (right) dorsal view of anal plate of codling moth. (pictures: Todd Gilligan, LepIntercept, USDA APHIS ITP, Bugwood.org)

Optionally, the mark release recapture method will be used to assess the extent to which SIT inhibits male orientation. The 1800 moths per ha released in each plot will be marked with a pink fluorescent dye. An additional 22 males marked with a green dye will be released in the SIT-treated and control plots. They will be released evenly from the same sites where the 1800 pink moths are released. The males marked with green will serve as proxies for a known density of "wild" males at a density that is 1/40th of that of the sterile males. Capture of males marked with the green dye in the monitoring traps will provide an assessment of treatment effect on male orientation. Catch and fruit injury data will be analyzed by appropriate statistics.

4.7. **Import of sterilized moths**

Permitting requirements will be different for each country. At a minimum an import permit would be required for each country and the Canadian partner may need an export permit issued before starting the shipments. In Italy and Greece, the authority that issues import permits is the phytosanitary office in agreement with the department of agriculture. In other countries, the relevant authorities might differ. A veterinary certificate may be required from some countries. It is recommended to directly contact personnel in charge of permitting. Personal contact gives the opportunity to directly answer questions that may come up and could help advance the permitting procedure. Once the import application/permits are acquired, shipping tests should be run as soon as possible to verify that the shipping process and routing works. Problems may arise at some point along the shipping route with inspection authorities or courier service. Once identified, these new issues will need to be resolved. It was suggested that the institution filling out the permit specify that the moths are sterile and are being used for experimental purposes. An application for experimental purpose is expected to have a better chance of success or be processed faster. A copy of the import permit (hard copy or PDF) should be given to the Canadian partner. Usually these permits are of limited duration (one year) and will need to be renewed whenever they expire. Phytosanitary and sterilization certification will be provided from OKSIR. The certification by OKSIR or any other Canadian authority should be placed together with the import permit in and on top of the package.

An additional step in the permitting process is to contact the potential shipping company to speak with the person in charge of live organism shipments and fill out any necessary related paperwork.

4.8. Quality control

Quality control (QC) measures are in place at the OKSIR facility in Canada that verify sterility and viability (Dyck 2010). Periodic tests should also be performed throughout the season by the recipient to measure possible impacts due to shipping and handling. QC tests should include flight ability testing based on the flying cylinder method (Carpenter et al. 2012) or another reliable simple method. Occasionally throughout the season, the following tests should be conducted: mating competitiveness, catchability, response to pheromone traps and sterility. It is recommended that a shared database is created where quality control test results can be posted by all collaborating countries and OKSIR so that QC data pre/post shipment can be compared. The database should include flight ability, sterility or any other remarks like damaged material or damaged packaging.

Prior to shipping complete shipments of live insects, trials would take place to check the process for import, transport and QC during shipping from Canada to collaborating countries.

5. Communications

Communications with all stakeholders is important to the success of a program that has a SIT component. Knowing your audience is important to how you frame the discussion. Explanations should be made easy to understand for the target audience without losing accuracy. Care should be taken to not assume that stakeholders understand scientific terminology and concepts.

5.1. Farmer Collaborations

Unlike trials that are using insecticides, released moths may leave the experimental block and could end up in monitoring traps of adjacent orchards. Therefore communication and education of local farmers and the monitoring agencies that check traps is important to avoid confusion and concern. Though there may be some potential negative impacts of area-wide IPM and eradication programmes using the sterile insect technique, the potential that the release of sterile codling moths could have a negative ecological impact is negligible (Nagel et al, 2005). This is because there are not residual effects on the insect (other than sterility): moths can be consumed by birds, bats, frogs etc. without any impact.

It should be explained to the farmers that sterile codling moths are obtained through irradiation of adult moths without the use of any GMO (Genetically Modified Organism) technology.

Discussions should be held with the farmers regarding the timing of the pesticide application in relation to the release of sterile moths. If possible, it is obviously best if pesticides are not applied within experimental blocks. If sprays (or other pest control tactics) are applied, they should be applied to both the control and the experimental plot. It is important to note that if Altacor/Coragen (Chlorantraniliprole) is used in the trial plots, male moth flight will be shut down due to an impact on wing muscles. Consideration should also be given to pesticide treatments directed at other pests or diseases like walnut husk fly, leaf rollers, leaf miners etc.

Farmers who are concerned about potential damage caused by released sterile females should be reassured by research showing that the level of radiation used is sufficient to consistently and reliably sterilize females (Bloem et al. 1999b). Periodic confirmation of female sterility is done following SIT protocols (Nagel et al. 2010). Petri dishes of sterile CM come with radiation level tags that are used by the OKSIR rearing facility, as well as the end user, to confirm that the moths have been exposed to the proper level of radiation.

5.2. **Public Relations**

A question that often comes up during conversations with the public is: "Are the sterile insects safe?" Conversations about radiation often require addressing misconceptions. A common misconception that has been expressed by the general public is the idea that the irradiated moths are radioactive. One way to help bridge this misconception is to remind them that they are exposed to radiation when they get X-rays at the doctor or dentist and they do not come home radioactive.

A public relations document could be produced after initial experiments are completed to advise department of agricultural staff/ leadership. The document may contain examples of earlier SIT successes including CM SIT.

6. Participation and Funding

6.1. Stakeholders

There are a number of stakeholders who could play a role in the research trials once a regional proposal is developed:

- FAO/IAEA
- Research institutions
- Universities
- National plant protection organisations
- Growers
- Industry
- General public/community

6.2. **Staffing**

It is important to have experienced research staff to lead and manage the implementation of the pilot trials. Occasionally growers can provide support to the project under supervision of the research staff.

6.3. Funding

A consortium could be established to facilitate obtaining EU joint funding to assist with financing the pilot trials.

In addition to the length of the project trial, funding considerations should include:

- Shipping arrangements and costs including size of the shipment container and weight
- Public relations communications efforts

7. Results

Expected outputs and outcomes could include:

- Measures of efficacy of the SIT under various experimental conditions.
- Proven efficacy may pave the way for various larger scale programmes in each country.
- Outcome leading to industry interest and development of benefit-cost analysis.
- Publication of results in scientific journals and outreach publications.
- Potential consortium interest for funding application.
- Identify potential obstacles (e.g. organic certification) and lessons learned providing basis for further improvement.

8. References

Adams, C. G., J. H. Schenker, P. S. McGhee, L. J. Gut, J. Brunner, and J. R. Miller. 2016. Maximizing Information Yield from Pheromone-Baited Monitoring Traps: Estimating Plume Reach, Trapping Radius, and Absolute Density of Codling Moth (*Cydia pomonella*) in Michigan Apple. J. Econ. Entomol. *In press*.

Bloem S., Bloem K. A., Carpenter J. E., Calkins C. O. 1999a. Inherited sterility in codling moth (Lepidoptera: Tortricidae): Effect of Substerilizing doses of radiation on field competitiveness. Envrion. Entomol. 24: 669-674.

Bloem S., Bloem K. A., Carpenter J. E., Calkins, C. O. 1999b. Inherited sterility in codling moth (Lepidoptera: Tortricidae): Effect of Substerilizing doses of radiation on insect fecundity, fertility and control. Ann. Entomol. Soc. Amm. 92: 222-229.

Bloem S, Carpenter J, McCluskey A, Fugger R, Arthur S, Wood S 2007. Suppression of the codling moth *Cydia pomonella* in British Columbia, Canada using an area-wide integrated approach with an SIT component. *In*: Vreysen MJB, Robinson AS, Hendrichs J eds. Area-Wide Control of Insect Pests. Springer Netherlands. Pp. 591-601.

Blomefield T., Carpenter J.E., Vreysen M.J.B. 2011. Quality of mass-reared codling moth (Lepidoptera: Tortricidae) after long distance transportation:1. Logistics of shipping procedures and quality parameters as measured in the laboratory. Journal of Econ. Entomol. 104: 814-822.

Carpenter J.E., Blomefield T., Vreysen M.J.B. 2012. A flight cylinder bioassay as a simple, effective quality control test for *Cydia pomonella*. J. Appl. Entomol. 136: 711-720.

Carpenter J.E., Blomefield T., Hight S.D. 2013. Comparison of laboratory and field bioassays of laboratory-reared *Cydia pomonella* (Lepidoptera: Tortricidae) quality and field performance. Journal of Appl. Entomol. 137: 631-640.

Cartier, L. 2014. A Benefit-cost analysis of the Okanagan Kootenay Sterile Insect Release Program. Okanagan College, Okanagan School of Business. Pp 26.

Dyck V.A., Graham S.H., Bloem K.A. 1993. Implementation of the sterile insect release programme to eradicate the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Olethreutidae), in British Columbia, Canada. In: IAEA Management of Insect Pests: Nuclear, and Related Molecular Techniques, IAEA-SM-327/29, Vienna, pp. 285–297.

Dyck V. A. 2010. Rearing Codling moth for the sterile insect technique. Food and Agriculture organization of the United Nations, Rome, Italy. Pp. 134-140.

(FAO STAT)Food and Agriculture Organization. 2017. http://www.fao.org/faostat/en/#data

Judd, G.J.R. and M.G.T. Gardiner. 2005. Towards eradication of codling moth in British Columbia by complimentary actions of mating disruption, tree banding and sterile insect technique: Five year study in organic orchards. Crop Protection 24: 718–733.

Klassen W, Curtis CF 2005. History of the Sterile Insect Technique. *In*: Dyck VA, Hendrichs J, Robinson AS eds. Sterile Insect Technique. Springer Netherlands. Pp. 3-36.

Lance D, McInnis D 2005. Biological basis of the sterile insect technique. In: Sterile Insect Technique. Springer. Pp. 69-94.

Nagel P., Peveling R. 2005. Environment and the Sterile Insect Technique. In: Dyck V.A., Hendrichs J., Robinson A. *eds* Sterile Insect Technique. Springer, Dordrecht.

Proverbs, M.D., Newton, J.R., Campbell, C.J., 1982. Codling moth: a pilot program of control by sterile insect release in British Columbia. Can. Entomol. 114: 363–376.

Voudouris C. Ch., Sauphanor B., Frank P., Reyes M., Mamuris Z., Tsitsipis A., Vontas J., Margaritopoulos J. T. 2011. Insecticide resistance status of the coding moth *Cydia pomonella* (Lepidoptera: Torticidae) from Greece. Pestic. Biochem. Phys. 100: 229-238.

Wise, J.C., A. H. Van Woerkom and L. J. Gut. 2015. Control of Codling Moth in Apple, 2014. Arthropod Management Tests 40 (1): A7 doi: 10.1093/amt/tsv007.42: 1348-1355.

9. Appendix

Appendix A: Theoretical predictions of the numbers of sterile moths required to suppress codling moth populations of different sizes.

Absolute densities of codling moth populations are estimated from pheromone trap catches according to methods outlined in Adams et al. (2017). This approach used a novel analysis of mark release recapture experiments to relate pheromone trap captures to absolute population densities of codling moth. This method uses 1 Pherocon II Delta Trap with L² tm codling moth lure to sample a 21 ha area. The table shows how these trap captures relate to population estimates per 21 ha and per ha. These estimates of absolute densities are used to estimate the number of sterile codling moth required per ha to reach the targeted over-flooding ratio or 40 sterile to 1 wild moth. Estimates from this method are not independent of trap density, meaning trap densities different to 1 trap per 21 ha cannot be estimated from this table.

Codling moth capture (1 trap/21 ha)	Population estimate (per 21 ha)	Population estimate (per ha)	Sterile moths required for 40:1 over-flooding
1	50	2	95
2	100	5	190
3	150	7	286
4	200	10	381
5	250	12	476
6	300	14	571
7	350	17	667
8	400	19	762
9	450	21	857
10	500	24	952
15	750	36	1429
20	1000	48	1905
25	1250	60	2381
30	1500	71	2857
35	1750	83	3333
40	2000	95	3810
45	2250	107	4286
50	2500	119	4762
55	2750	131	5238
60	3000	143	5714
65	3250	155	6190
70	3500	167	6667
75	3750	179	7143
80	4000	190	7619
85	4250	202	8095
90	4500	214	8571
95	4750	226	9048
100	5000	238	9524

Appendix B: Contact List

Rui Cardoso Pereira
Head - Insect Pest Control Section
Joint FAO/IAEA Division of Nuclear
Techniques in Food and Agriculture
Vienna International Centre, PO Box 100
A-1400 Vienna, Austria
R.Cardoso-Pereira@iaea.org

Marc Vreysen
Head - Insect Pest Control Laboratory
Joint FAO/IAEA Division of Nuclear
Techniques in Food and Agriculture
Vienna International Centre, PO Box 100
A-1400 Vienna, Austria
M.Vreysen@iaea.org

Cara Nelson
Okanagan-Kootenay Sterile Insect Release
Program
1450 K.L.O. Road, Kelowna, BC, V1W 3Z4
CANADA
cnelson@oksir.org

Larry Gut
Chris Adams
Michigan State University
Center for Integrated Plant Systems
578 Wilson road, room 106
East Lansing, MI 48824 USA
gut@msu.edu
adamsch@msu.edu

Sylvia Bluemel
Institute for Sustainable Plant Production
(NPP)
Spargelfeldstr. 191
A-1220 Wien AUSTRIA
sylvia.bluemel@ages.at

Johannes Jehle
Julius Kühn-Institut (JKI)
Federal Research Centre for Cultivated Plants
Heinrichstr. 243
64287 Darmstadt, Germany
Johannes.Jehle@julius-kuehn.de

Panagotis Milonas
Department of Entomology
Benaki Phytopathological Institute
8 S. Delta, 14561 Kifissia, Greece
p.milonas@bpi.gr

Phillipos Ionnadis
Eurofarm SA
3rd Klm OnR Alexandria-Veria
593 Alexandria, Greece
filioan@otenet.gr

Claudio Ioriatti
Institute CREA-FRU,
via Fioranello 52, Rome, Italy
claudio.ioriatti@fmach.it

Massimo Cristofaro
Center for Technology Transfer
FEM- IASMA
v. E.Mach, 1
38010 S.Michele a/A, Italy
m.cristofaro55@gmail.com

Charles Whitfield
Pest and Pathogen Ecology for Sustainable
Crop Management
NIAB East Malling Research
New Road
East Malling
Kent ME19 6BJ UK
Charles.Whitfield@emr.ac.uk

Agnès Verhaeghe
SENURA - Scientifique et Technique Fruits et
Légumes (Ctifl)
Les Colombières
38160 CHATTE, France
averhaeghe@senura.com

Herman Helsen
Wageningen Plant Research
Lingewal 1
6668LA RANDWIJK
Netherlands
herman.helsen@wur.nl

Appendix C: Country proposals

ITALY

Control of codling moth in Italy with sterile insect technique: pilot experiences in two locations.

A first field trial will be carried out in Trentino (northern Italy), in a 16 ha organic apple orchard where CM is currently controlled with mating disruption supplemented with CpGV. The orchard area will be divided in 5 blocks: in two blocks (Block 1 & 2), the SIT will be implemented in conjunction with mating disruption that is applied as a routine control tactic by the farmer. In two other blocks (Block 3 & 4), only mating disruption will be implemented without the use of SIT. The two experimental treatments will be separated by a buffer zone of at least 100 meter (Block 5). From the end of April to the end of September, sterile moths will be released weekly in the two SIT blocks at a rate of 1800 moths/ha. Moths will be released in batches of 200 from each of 9 locations/hectare. Within the plots, release sites will be interspaced by a 33-meter distance.

A similar experimental design will be repeated in a smaller orchard near Rome where an SIT plot (2 ha) will be compared with an untreated control plot (2 ha). A buffer zone of 3 ha will separate the two experimental treatments.

The efficacy of the SIT will be estimated by the ratio of the number of wild males vs. sterile ones (marked) in pheromone traps, fruit infestation at the end of the season, and counts of the overwintering larvae using cardboard bands.

Monitoring of the adult CM population will be done with six Delta-style sticky traps baited with standard pheromone lures that will be deployed in the upper canopy in the central area of each of the four blocks and checked weekly throughout both CM flights.

Fruit injury will be assessed by inspecting 1000 fruits per blocks (20 from each of 50 trees) for the presence of CM infestation (stings or frass).

Overwintering population of the two experimental treatments will be estimated by installing 40 cardboard bands in each block before the end of July. The cardboard will be collected and inspected during the winter for counting the overwintering larvae. Reduction of catch and fruit injury data over the generations will be analyzed with appropriated statistical analyses.

GREECE

Motivation: codling moth is an important pest for apple production in Greece. Farmers principally rely on insecticide applications but there is growing evidence of resistance development to available insecticides (Voudouris et al. 2011). In addition, there are indications based on field applications that the new products are not as effective as they were a few years ago. Mating disruption is available for CM management but with relatively limited adoption by the farmers. There is expressed interest by the farmers' cooperatives for new sustainable solutions and by private entities that are interested in pome production and crop protection such as Eurofarm S.A. and Novacert S.A. The involvement of the Benaki Phytopathological Institute in the project ensures the general interest of a research institute that is committed to provide sustainable solutions to the Greek farmers. In addition, there is growing interest from the local and international markets for limited residue levels and even absence of detectable pesticides.

Challenges: The first challenge would be the approval of an import permit for live insects for experimental purposes through the Ministry of Agriculture. Secondly, we need to explore the obstacles that could appear during the transport of the moths from Canada all the way to the field. Cooperation with the farmers would probably be another challenge as it is a totally new approach for them and they have limited experience with techniques other than pesticides application.

Because of the small size of the fields in Greece the application of the SIT method needs the collaboration of two or more farmers simultaneously to have one acceptable plot size and the challenge will be the communication and cooperation with them. Another challenge we anticipate is the length of the flight period of CM in Greece. CM completes 3 generations starting from May until end of September.

Funding: in Greece we are going to explore the potential for national funding of a project. Basically, we will seek the opportunity through the General Secretariat for Research and Technology and through the Rural Development Fund. The first instrument would be an open call for research proposals and it is expected to open soon. We believe that we will have a good possibility to get the project funded because it will be a collaboration of public and private sector. The second option is expected to open at the end of summer and supports innovative ideas in agriculture in cooperation of farmers unions and research entities. Another possibility would be to get some support from the cooperatives or from the regional administration. Of course we would be interested in an international consortium that would seek funding for an international project.

GERMANY

Motivation for SIT trial in Germany

In Germany, apples (31,500 ha) and pears (1,950 ha) are the most widely grown fruits. Larvae of codling moth are the most severe insect pests for apple and pear production and, if not controlled, cause high economic losses to growers. Depending on the weather conditions, CM has one or two generations in Germany. Different chemicals, pheromone-based mating disruption and the application of CpGV products are available for integrated and/or organic pome fruit production. Whereas pheromone-based mating disruption is applied on about 10% of the growing area, CpGV products are being used on about one third of the planted area. Mating disruption and CpGV products remain indispensable for the organic production of apples and pears.

In view of the necessity to reduce the risks caused by chemicals, the increased removal of insecticides from the market by national governments and the EU, emerging resistance to insecticides and to CpGV products, there is an obvious need for more control tactics suitable for integrated and organic production. The SIT may provide a new option for an environmentally sound control of codling moth, especially where currently applied control methods have failed to provide sufficient codling moth control. Therefore, it is planned to test the feasibility and efficacy of SIT in selected orchards. Julius Kühn-Institute (JKI) is interested to carry out and to coordinate field experiments in collaboration with partners from local extension services on an area of 4-8 ha starting in 2017 or 2018.

UNITED KINGDOM

There are two proposals to develop. The first, a small scale trial added to a project currently running on mating disruption in the UK. There is the potential to incorporate a trial in 2017 or 2018 assessing the combination of mating disruption + SIT. This would allow some preliminary data to be gathered, test the import and transportation of sterile codling moths from Canada, and test field trial protocols. The funding, partners, field sites, and staff are already in place for this work. It however will require an agreement from all partners.

The second proposal to develop is for a larger (3 year) project to carry out a thorough assessment of the suitability of SIT in the UK tree fruit industry. Potential funders include Levy body (AHDB), producer organizations (e.g. Avalon ltd.), marketing companies (e.g. WorldWide Fruit), advisory boards (ADAS), and possibly the Department for Environment Food and Rural Affairs (DEFRA), and a SIT commercialization partner (e.g. Koppert, Syngenta, Russell-IPM). The proposal should aim for work starting in 2018 or 2019. The main aims of the work would be to: 1, compare SIT and/or SIT + other compatible methods (e.g. mating disruption, CpGV) to conventional chemical insecticides; 2, assess impact on beneficial insects; 3, assess impact on other and secondary pest; 4, carry out simple economic assessment; 5, small assessment on grower response to using SIT; 6, collaborate with other European partners and/or share and compare results.

NETHERLANDS

The Dutch partner Wageningen Plant Research will apply for a national or regional grant for executing the research work. The first field experiment is expected to start in 2018. Logistical and regulatory aspects of the work will be explored in 2017.

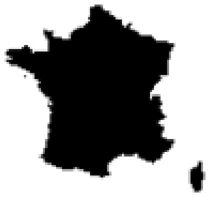
Biology of the pest

In the Netherlands codling moth develops one complete generation per year and a second incomplete generation in some years. As a result of climate change, the frequency of years with a second generation strongly increased over the past decades. Experience shows that an effective control of the first generation makes control measures against the second generation superfluous.

Test location and field test

The field work would encompass field tests on one or more private farms. In the first year we envisage working on an organic farm with a moderate, known codling moth infestation level. The farm consists of two orchard blocks, each of about 6 hectares, 300 meters apart. During the first flight period moths will be released in one of the orchards, in accordance with the common research protocol. The grower will apply pheromone mating disruption or Cp GV in both fields. All other insecticide applications will be the same in both fields. Efficacy of SIT will be measured with pheromone traps and by counting damaged fruits before harvest.

We will seek cooperation with a commercial partner locally in order to explore the potential for further development and commercialization of SIT of codling moth.



FRANCE

The first step should be to have the authorization (permit) to import the sterile moths from Canada and to organize the shipping. As soon as authorization is obtained, we will organize the first shipment, to verify if all steps will progress smoothly. The first shipments will be used to carry out quality control (flight ability, sterility, release and recapture).

At the same time, we will look for an orchard where percentage damage and management of the previous year is known. The orchard will be used to assess the efficacy of the SIT, once we have signed the agreement with the farmer concerned about this trial (management of the buffer/ choice of product against Walnut husk fly)

Modalities : M1: CpGV; M2 : SIT
Normally, it will be necessary to have moths from the beginning of May until the first week of September (around 20 weeks). If our plot is 4 hectares, we need 10 dishes by week, 200 dishes for one plot for one season. A plan of the orchard and position of the release will be determined.
Observations :
\
Monitoring with traps in the SIT site and in the control site (Five traps /plot)

- o Local weather

Damage on the tree and damage at harvest (20 Fruits/tree and 50 trees by trial (border ¼; Centre ¾)
Comparison between both programs:

- Limits of the SIT/ cost
- Advantages of SIT
- Build a programme to follow by a company to organize the development if the method should be developed.

If the cost is more for SIT, with a few trials we will have to work with local organization to help the grower try this solution.

Timelines:

March/ May:
- Authorization
- Selection of the orchard

May/September
- Shipment and release
- Trap monitoring

End of G1, end of G2 Harvest:
- Count of damage

November/December
- Synthesis
- Meeting to exchange information about our trial

Develop funding proposal beyond the three year trial:
- Cost of each trial
- Time necessary
- Organization of our group

- Publications of our results

AUSTRIA

With regard to the obligatory implementation of the EU Directive 2009/128/EC on Sustainable Use of Pesticides, the decreasing availability of conventional insecticides and the public concern of pesticide use in agriculture, the need for innovative, alternative plant protection measures and tools has been generally recognized in Austria.

Pome fruit production, especially apple production is the most important fruit crop production in Austria. Due to an increase in the area of organic apple production, and as CM belongs to the most important arthropod pest in apple production in Austria (with varying importance over the last decades) SIT is considered a promising additional or alternative method for CM control.

Before field trials as indicated in Table 1 can be started (earliest 2018), several preparatory tasks to close information gaps have to be accomplished. These comprise communication and raising awareness with various stakeholders, especially growers, local advisors (especially agricultural chambers or growers associations), retailer organisations (farmers have to fulfil their contracts and take care of their additional regulations) and probably specific NGOs. A decision about which treatments will finally be tested could only be made after a first communication to growers and local advisors. AGES (Agentur für Gesundheit und Ernährungssicherheit) could be involved in the preparative communication process, in the clarification of national legal aspects/procedures, in the identification of partners for the field trials and in the planning and supervision of field trials.

Additionally information about national and transnational (European) funding options could be compiled (see also slides).

UNITED STATES

CM is the principal pest of apple that Michigan (MI) growers must control to produce marketable crops. Without effective control, losses can exceed 50% of the crop. Managing this key pest has become quite challenging for MI fruit growers due to loss of compounds through restrictions or resistance, the high cost of new insecticides, concerns about worker safety and the public's interest in reducing the use of insecticides. The sterile insect technique is a promising option for suppressing populations of CM.

The Michigan State University Tree Fruit Entomology team has been working with the British Columbia SIT program for the past five years, importing sterile CM and releasing them in Michigan apple orchards to investigate the response of CM to pheromone-baited traps. Growers participating in our research project have noted that CM densities are greatly reduced in orchards where we have released moths, often to the extent that insecticide sprays are no longer needed.

The OKSIR Programme has initiated collaborative trials whereby growers outside of Canada would have access to sterile moths for managing CM. The aim is that sterile males could be used much like pheromone-based pest management. A permit to receive OKSIR moths from Canada has been applied for and is working its way through the regulatory system. We anticipate being able to initiate research trials in 2017.

Year 1 field trials in Michigan will focus on testing the effectiveness of mass release of sterile moths for managing CM in commercial orchards. Mass-reared CM irradiated with 200 Gy will be imported from the OKSIR facility in Osoyoos, British Columbia, Canada. Over the past four years, we have obtained millions of sterilized moths from this facility. Moths will be shipped overnight in coolers and released in orchards within six hours of receiving them.

Grants have been submitted to grower and state funding agencies. We propose to evaluate the efficacy of two sterile release programmes:

1) release of sterile moths plus judicious use of insecticides as needed or
2) pheromone-based mating disruption overlaid with the release of sterile moths plus judicious use of insecticides as needed.

Control treatments will be included in which insecticides only or mating disruption plus insecticides will be used for CM control, i.e. no sterile male releases. All research will be conducted in commercial apple orchards in regions where CM is most problematic: Southwest, Southeast, or north of Grand Rapids in the region commonly referred to as The Ridge. Field plots will consist of 10-30 hectare commercial apple orchards subdivided into smaller plots. The study will be set up as a randomized complete block design with one replicate of each treatment within a given farm. There will be a minimum of 120-meter buffers between the treatment blocks. Insecticide sprays will be applied for pests other than CM as needed to protect the crop from commercially unacceptable damage. Release protocols and data collection will be as generally proposed in this document.

We also plan to conduct studies to determine the optimum density and frequency of release of sterile moths for managing CM, potentially improving the economics and enhancing the prospects for adopting this technology. This will entail studies in which moths are marked with different colored dyes and released at various over-flooding ratios or distribution patterns.