Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture

AFRICA, ASIA, LATIN AMERICA

Research into fruit fly taxonomy adds knowledge of cryptic species on the family tree

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Getting to know members of the Tephritid family can be quite a colourful experience. Tephritidae is the family name of fruit flies, one of the most damaging insect pests of horticultural products worldwide. Over the centuries, taxonomists have broken the Tephritidae into some 500 genuses and then further into 5 000 species – most of which have colourful wings, interesting mating rituals and territorial personalities. But today, for some species of economic and quarantine importance, questions arise as to whether taxonomists have been correct in how they have identified the species – whether they are actually distinct biological species or variants of the same species. The Joint FAO/IAEA Division conducted coordinated research aimed at resolving some of the taxonomic questions surrounding major "cryptic" species complexes, meaning questions about closely related species within a genus. The study led to accurate taxonomic alignment of the species and provided valuable information needed for effective application of the sterile insect technology (SIT) and facilitation of international trade.

This is the story of three fruit fly complexes and a suspected complex of significant economic importance. These pests, all of which can cause major yield losses and by their very presence keep a horticultural sector from exporting, are known scientifically as *Anastrepha fraterculus* (South American fruit fly complex), *Bactrocera dorsalis* (Oriental fruit fly complex), *Ceratitis* FAR (fruit fly complex from the African region), and *Zeugodacus cucurbitae (formerly Bactrocera cucurbitae*), the melon fly suspected complex.

Accurate fruit fly taxonomy makes it possible to assess which species are present or absent in a given area which, in turn, provides a scientific basis for countries to set up their import regulations according to international phytosanitary standards, and to develop appropriate and effective fruit fly surveillance and control methods. For example, using the sterile insect technique (SIT), a species-specific and cost-effective environmentally friendly method of controlling fruit flies, requires a substantial initial capital investment. Thus, before embarking on such an endeavour, it is essential for governments and industry to know exactly which species are present in the area of interest.

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For example, over the years, taxonomists identified and named several closely related species of *Bactrocera dorsalis*, to a point where it became the "dorsalis complex". The *Bactrocera* species are native to Southeast Asia, but when one of its species arrived in East Africa from Sri Lanka in 2003, taxonomists identified it as a new species, naming it "*B. invadens*" because it spread quickly and aggressively across more than ten African countries. In response, regulatory authorities of importing countries required exporters from the African countries to certify that their horticultural produce originated from *B. invadens*-free areas or that the produce was free of the pest – but this required stringent post-harvest treatments that were not readily available for this supposedly new species. As a result, the exporting African countries dealt with years of trade restrictions that seriously affected their mango and banana exports.

The Joint Division, with participating scientists from 20 countries, decided to take another look at the fruit fly complexes, including the case of *B. invadens* and other suspected species. With the availability of new, more precise tools to identify species, the scientists began the processes of comparing morphology, e.g. of wings, antennae and organs; checking at the genetic level for consistency; and checking propensity for mating compatibility, all of which are accepted designations for identifying species.

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Study tunes in on what may be evolution in the making

With research going on in several laboratories, the researchers reached extremely valuable conclusions. For example, they found the *B. invadens* and *B. dorsalis* were morphologically and genetically close and mating propensity was high. In other words, they proved these two species were actually the same *dorsalis* species – *B. invadens* did not really exist as a separate species. This insight will now enable the African fruits growers and export industry to use technologies already available for pre- and post-harvest control of *B. dorsalis.* In fact, the researchers determined that four of the species in the dorsalis complex – *B. dorsalis, B. papayae, B. philippinensis and B. invadens* – were all the same biological species. Looking forward, this means that growers and industry will be much better able to target their control programmes and provide phytosanitary certification to importers.

The research conducted on the *Ceratitis* FAR complex from the African region identified five different species. *C. capitata, C. fasciventris, C. anonae* and *C. rosa* were confirmed as individual biological species thus, for the most part, Ceratitis' taxonomy remains unchanged, although they did identify a new species related to *C. rosa.* – It will be known simply as R2 until taxonomically described and a proper Latin name has been chosen. As for the *Zeugodacus cucurbitae,* it was concluded that the melon fly does not represent a cryptic species complex in regards to its geographic distribution or its host range.

And finally, the study of the *Anastrepha,* specifically the South American fruit fly complex and its one species, the "*fraterculus*", proved quite surprising. Four morphotypes with their geographical distribution were defined as "distinct biological species". This might be an indication that the fraterculus complex has been in the act of separating into distinct species, and that the researchers have actually tuned in during its process of evolution. As a follow-up, scientists will meet again in 2016 to sort out issues on three Brazilian morphotypes and the description of the new species, and to choose a name for the new "*fraterculus*".

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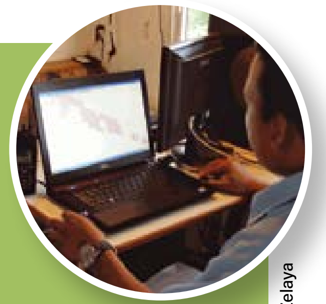

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USA: CALIFORNIA AND FLORIDA

Prevention rather than crisis reaction protects US horticulture industry from medflies

As far as the horticulture industry is concerned, one piece of fruit in a traveller's backpack or a shipment of fruit arriving at a seaport both portend disaster if the fruit also contains hitchhiking larvae of the Mediterranean fruit fly (medfly) – larvae that could escape inspection at port of entry and initiate an infestation. In the past, California and Florida, the main USA horticulture-producing states, used a reactive approach to control medfly outbreaks, such as ground and aerial insecticide-bait spraying – but this was only partially effective and there were concerns about negative public health and environmental impacts. In the mid-1990s, at the recommendation of a technical advisory committee in which staff of the Joint FAO/IAEA Division had a leading role, California and Florida both initiated the sterile insect technique (SIT), calling for area-wide preventive and continuous aerial releases of sterile male medflies over high-risk areas – an endeavour that has substantially reduced overall cost and prevented establishment of the pest.

Until the mid-1990s, California and Florida controlled medfly outbreaks mainly through using insecticide-bait sprays. Yet, checks of surveillance traps increasingly found adult flies, indicating new infestations of the pest. The inadequate results of the reactive approach and concerns about its negative environmental and health effects led the two states to initiate a preventative medfly control operation – the sterile insect technique (SIT) – with the advice and technical guidance of the Joint Division.

SIT calls for rearing, sterilizing and releasing an enormous number of male medflies near where an infestation is or might occur. The males mate with wild females but there are no offspring. In the case of California and Florida, setting up a preventive SIT programme, and ensuring the weekly availability of millions of sterile medflies to release over the large areas at risk proved to be a challenge. The solution came from the largest insect rearing facility in the world – the El Pino facility of the Moscamed Programme in Guatemala. Now, the hundreds of millions of sterile male insects needed to effectively cover the areas at risk are shipped weekly from Guatemala and delivered for release in the two states.

When California and Florida used a reactive approach – with increased insecticide-bait applications – medfly outbreaks were becoming ever more frequent and severe

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as a result of more infested fruit entering the states. It meant exporters often faced costly quarantine restrictions from countries that only accepted shipments from medfly-free areas. Also at that time, insecticide-bait-based control programmes that used broad-spectrum organophosphate insecticides, such as Malathion, were facing serious public opinion opposition. In urban areas of Los Angeles, residents were extremely concerned about the insecticides wafting onto public property as well as their lawns and cars.

SIT is cost effective, safe and efficient

In researching the situation, studies determined that the reactive insecticide-bait approach in California had a direct average cost of approximately US$33 million a year, which included the insecticide and labour costs, but mainly represented market losses due to quarantine restrictions. They also determined that a preventive SIT approach, calling for continuous release of sterile male flies over targeted high-risk areas, would have an overall annual cost of approximately US$13 million for weekly shipments of the sterilized male medflies from Guatemala, the packing, holding and aerial release, and associated labour costs. Not only were expenses cut by more than half, there would be multimillion dollar savings by avoiding restrictions to trade of horticultural products.

Seeing the financial, human health and environmental advantages, the departments of agriculture and the horticultural industries of California and Florida adopted the preventive SIT approach. The numbers vary according to the pest situation, but in California, this calls for releasing from 30 to 150 million sterilized male flies each week over an area of up to 6 500 km^2. In Florida, it is 25 to 100 million flies each week over an area of up to 1 640 km^2. In addition to reducing cost, it is safer – with greater protection to human health and less impact on the environment due to reduced insecticide residues.

The SIT preventive pest control is a suitable strategy to protect pest-free areas from the entry and establishment of insect pests. And, because the sterilized males will seek wild females upon release, SIT is an ideal preventive control method for a wide area that has multiple potential pest entry points. SIT also relies on surveillance traps to provide on-the-ground early detection and to assess effective eradication of small populations in outbreaks.

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MEXICO, GUATEMALA, USA

At Mexico-Guatemala border, sterile insect technique has controlled the northward march of medflies since 1982

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Imagine an area of nearly 200 000 km² that needs to be monitored on a daily basis – monitored for the presence of a destructive insect smaller than a fingernail. It has been more than four decades since the governments of Mexico, Guatemala and the United States agreed to work together to stop the spread of the Mediterranean fruit fly – an agreement which evolved from three bi-lateral agreements signed in 1975 and 1981 into the one single Trinational Moscamed Cooperative Agreement signed by the three governments in 2015 plus a memorandum of understanding with Belize. In their years of working together, the countries have kept the medflies out of their orchards and fields but also, with the support of the Joint FAO/IAEA Division, have continued to improve the technology, including the use of the sterile insect technique (SIT), insecticide-bait applications and on-the ground surveillance. It is all done with annual financial contributions by the three countries and significant numbers of trained personnel who work in the mass-rearing and sterilization facilities and in the field to keep the four countries safe from what could amount to billions of dollars in annual damages to production and trade – if the insects were allowed a foothold.

No one really knows for sure when or how the medfly began its odyssey from its original homeland in East Africa to become one of the most reviled and feared insect pests on the planet. Theories include Ethiopian coffee traders who inadvertently took the flies to North Africa, and sailors who purchased fly-inundated citrus fruits in North Africa and brought them to Europe.

As for the Americas, medflies arrived in Brazil in 1901, possibly through trade with Portugal and, in subsequent years, invaded most of South America. In 1955, they reached Costa Rica in Central America, possibly through trade with West African colonies, and then spread to southern Guatemala in 1976, on a northward trajectory that put the high value fruit and vegetable industry of Mexico, the United States, Guatemala and Belize in their sight.

At the same time, the Joint Division was developing and testing a daring method for medfly control. Known as the sterile insect technique (SIT), it had been used since the 1950s for the suppression and eradication of screw-worm flies but never for medflies. The Joint Division initiated research to use the SIT technique for medflies at its laboratories in Seibersdorf, Austria, where it reared and sterilized the number of insects needed for field testing. In 1969, pilot testing was undertaken on two Mediterranean islands off the cost of Italy, Procida and Anacapri, releasing the sterilized male insects to mate with wild females – and thus produce no progeny. The pilot was declared a success in 1971.

Although still a visionary idea, by the time medflies were detected at the border of Guatemala and El Salvador, the countries were able to transfer the technology from Seibersdorf and adopt it in an attempt to quash potential disaster. A medfly mass rearing and sterilization facility was constructed in Metapa, Chiapas, in southern Mexico, with the first sterile fly releases in 1978. Four years later, after releasing billions of sterilized flies, the medfly was proclaimed eradicated from approximately 1 million

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hectares of Chiapas, a state on Mexico's South Pacific Coast that borders Guatemala. Years later, a second and larger rearing facility, El Pino, was constructed in Guatemala, which greatly contributed to the goal of protecting the medfly-free areas in Guatemala and preventing the northern spread of the pest.

Building from success, focus switched from eradication to maintaining a containment barrier on Guatemalan territory away from the Mexican border and to the gradual medfly eradication from Guatemala. Today, that biological barrier has served its purpose and the programme continues to release sterilized male medflies – as many as 1.3 billion each week – to maintain it.

Technological advancements bring down SIT costs and increase impact

Over the decades, improved technology has increased efficiency and brought costs down. Joint Division researchers developed a medfly strain that only produces males, so instead of rearing, sterilizing, packing, transporting and releasing both males and females, the programme only produces males. This not only substantially cuts costs, it increases the induction of sterility in the population by forcing sterile males to seek wild females.

On-the-ground surveillance is regarded as the eyes of a programme. Baited traps are set and, when checked, they tell programme managers and growers if there are medflies in the area, where they are and how many – critical information for where, when and how many sterilized flies to release. Traditionally, traps baited with parapheromone lures were set to attract males. But in the early 2000s, the Joint Division supported the development of a female biased trapping system using food attractants with a mix of the proteins females need to mature their eggs. The females tend to respond earlier to the protein lure than the males respond to the parapheromones, which gives more advanced warning of need to step up control. In addition, a recently developed organic and environmentally friendly insecticide can be used for effective medfly control over large areas prior to the targeted release of sterile male medflies – the step that leads to eradication of the medfly population.

Today, the facilities in Mexico and Guatemala rear and sterilize over 1.5 billion medflies per week and have expanded their production to other species of fruit flies of economic significance. Keeping the USA and Mexico medfly free has created conditions for the development of their multibillion dollar horticulture industries and has paved the way to increase production and export of fruits and vegetables from Guatemala and Belize.

This long lasting collaboration with the Joint Division resulted in the recognition of the Moscamed Programme as a Collaborative Centre of the IAEA in 2010. Through this partnership, harmonized SIT and associated technologies continue to be transferred to hundreds of technicians in countries that participate in international training courses hosted by the programme, and the technologies have been advanced through joint research and development.

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COSTA RICA

Stinging wasps replace chemical pesticides becoming control agents in fighting stable flies

Costa Rica's position as the world's largest producer of pineapple brings with it a parallel problem for the country's livestock and dairy industry. Its pineapple processing plants are surrounded by mountains of pineapple residue, and that residue provides a breeding ground for the aggressive, blood-sucking "stable fly", a fly that can wreak havoc on cattle and affect their productivity. In an effort to control the fly without resorting to chemical sprays, the Costa Rica Institute of Agricultural Technology Research (INTA-MAG) with the support of the Joint FAO/IAEA Division identified another way to do the job – using a wasp. Not just any wasp. This wasp is a "biocontrol agent", a natural enemy of the stable fly that does no harm to the environment. The extensive experience of the Joint Division in the use of irradiation in biocontrol and in insect mass rearing has made this an ideal partnership.

The stable fly got its name from its habitat. Known scientifically as *Stomoxys calcitrans*, the stable fly hangs out almost anywhere that horses, cattle and other agricultural animals can be found. A bit smaller than the common housefly, its stinging, blood-sucking bite stresses the dairy and livestock cattle it attacks and can lead to anemia, weight loss and reduced milk production.

Costa Rica's dairy and livestock sector has a particular problem with the stable fly because of the country's position as the world's largest producer of pineapple. The stable fly lays its eggs in pineapple residue and, when the new flies emerge, they fly off in the direction of the nearest cattle or dairy farm. Efforts to control the stable fly with pesticides can have public health or environmental impacts.

Now, there is a new player in the control game, the *Spalangia*, a tiny parasitoid wasp. Parasitoid, not to be confused with parasite, refers to an insect that attacks other insects, which is exactly what this little wasp does. It lays its eggs in stable fly pupae. Upon hatching, the wasp larvae feed on their host – they consume the stable fly pupae. This means that the wasp is born but the stable fly never emerges. It all happens naturally, hence the *Spalangia* is called a "biocontrol agent".

The Joint Division has provided scientific support in the development and use of nuclear techniques to enhance biocontrol against insect pests. For example, it supported

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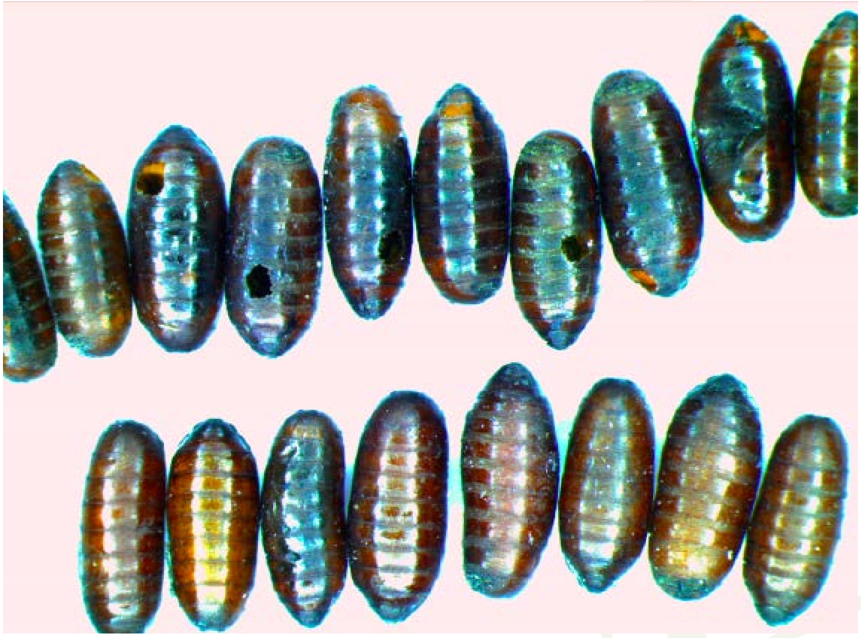

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the Moscamed Program in Mexico in developing the mass production and release of other wasp species to control fruit flies that threaten the country's horticulture sector. Now, in supporting the Ministry of Agriculture (MAG) of Costa Rica in adapting the methodology for stable flies, it facilitated the establishment of a facility to rear the needed numbers of *Spalangia*, allowing testing of the method at pilot level in dairy and beef farms.

Irradiating stable flies provides extra safeguard for wasp release

In order to make this happen, researchers from INTA-MAG, with support of the Joint Division, rear stable flies and wasps side-by-side. This method requires bringing a colony of stable flies into the facility and using its pupae to rear the wasps. When the wasps become adult, they are released in areas where pineapple processing facilities are in proximity to dairy and beef farms. Once the process is started, the wasps naturally seek out stable fly pupae to lay their eggs, so the process of controlling the stable flies continues quite naturally.

There is also a prequel to this scenario. When the INTA-MAG laboratory rears the wasps using the stable fly pupae, it cannot be sure that wasps will lay their eggs in every single pupa, meaning there is a chance that some stable flies themselves will actually emerge. Thus, the researchers use gamma rays to irradiate all of the stable fly pupae before the wasps come to lay their eggs. That way, even if a few stable flies emerge, they will be sterile, so no progeny, which further assures an effective biocontrol programme.

This has proven to be a safe and cost-effective approach, reducing the need for potentially dangerous and costly chemical insecticides. In addition, animal health, and milk and meat production improve when they are no longer under attack by stable flies. Building on its successful implementation, the Costa Rican Ministry of Agriculture is putting the information produced and techniques developed into a national action plan to suppress stable fly infestations in affected areas throughout the country. Looking ahead, the stable fly is found worldwide and breeds on residues other than pineapple, meaning there are many countries that will be able to take advantage of this methodology for pest control. And as for *Spalangia*, they neither sting nor bite livestock or humans.

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