Suppression of Mediterranean Fruit Fly Using the Sterile Insect Technique In Neretva River Valley of Croatia

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Abstract

Background: Mediterranean fruit fly, *Ceratitis capitata,* is a pest of high economic importance in Croatia attacking several cultivated (*Prunus armeniaca, Prunus persica, Prunus domestica, Ficus carica, Malus domestica, Pyrus communis, Citrus reticulata, Citrus sinenssis* and *Dyospiros kaki*), wild (*Arbutus unedo* and *Ficus carica*) and ornamental host plants (*Eriobotrya japonica, Fortunella kumquat, Feijoa sellowiana* and *Citrus aurantium*). In Neretva valley, *C. capitata* affects production of mandarins, *Citrus reticulata* mainly for export to EU and Russia, with annual yield valued to over 25 million euros. Beside infestation in mandarin fruits, medfly also cause problems to exports due to quarantine and food safety regulations. After conducting economic and technical feasibility studies, two years of successfully suppression trough an Sterile Insect Technique (SIT) pilot project, the Croatian Ministry of Agriculture expand the project to the lower part of the Neretva valley, covering over 4000 hectares of fruit orchards, mainly mandarins. Since year 2013, grower associations from Neretva valley representing producers and industry joined to the project funding in terms of supporting part of the costs of labour, energy and sterile males. *Methods:* Fly emergence and release facility was build and equipped in the city of Opuzen, with current packing capacity of up to 30 million of sterile flies per week. Releases of sterile males are performed mainly with two ground release machines mounted on vehicles, using chilled flies. Trapping system is set and geo-referenced over the whole SIT treated (4000 ha) and non-treated area of the valley (additional 4000 ha) with Tephri Traps using 3component lures (ammonium acetate, trimethilamin and putrescin) as attractant. Captured flies are checked using fluorescent lamps to separate sterile from the wild flies and to provide information on the insect population levels. Routine fruit sampling is undertaken to evaluate fruit infestation and the consequent efficacy of the suppression methods.
Results: Results of the application of sterile insect technique after two years of pilot project and during two years of suppression project showed that medfly population, measured as number of larvae per kg of fruit was significantly reduced. In comparison to non-treated area in the upper part of the valley, medfly population was reduced by 92.4% in figs, 73.9% in peaches and 96.8% in mandarins during 2012 and by 100% in figs, 57.3% in peach and 96.7% in mandarins during 2013. *Conclusions:* The aim of the project is to extend to the all production area of the valley and engage on aerial releases to target the whole population of the pest and to improve the effectiveness of the sterile insects.

Key words: *Ceratitis capitata*, mandarins, international trade, sterile releases

Introduction

The Mediterranean fruit fly - *Ceratitis capitata* Wiedemann (Diptera, Tephritidae) is a pest of great economic importance in the Neretva valley, affecting production of several important crops. Its negative effects are manifested mainly in the export oriented production of mandarins *Citrus reticulata* Blanco. The Mediterranean fruit fly in the Neretva valley was first detected in 2001 (Pelicarić et.al. 2001, Pelicarić & Bjeliš 2002, Bjeliš & Pelicarić 2004). It was estimated that the damage this pest causes to the mandarin production in orchards as well as the economic damage observed in the export activities reach to the total of 10 to 30% depending mainly on weather which affect the pest incidence and intensity (Bjeliš et.al., 2007).
The Neretva valley extends to 15 000 hectares of Croatian territory, of which 7 000 hectares are agricultural areas, where the mandarins most economic important agricultural production. Mandarin is one of few fruit species in Croatia which production quantities suffice the needs of the domestic market and 75% of total annual yield is exported mainly to countries of former Yugoslavia, the EU and the Russian Federation. Total annual production of mandarins in the Neretva valley, varies between 50.000 to 80.000 tons and increases each year due to many young plantations, with an estimated annual production over 120,000 tons in five years.

In order to organize and implement legal measures for the suppression of the Mediterranean fruit fly, various analysis were conducted to define and adopt the most efficient control (Bjeliš, 2004). With previous studies of economic and tehnical feasibility it was recognized that the Sterile Insect Technique (SIT) is the best method for suppression of the Mediterranean fruit fly in the Neretva vall all over the textey for several reasons. The cost-benefit analysis resulted in C:B = 1:6, meaning that one invested Euro produces six times more benefits (Bjeliš, 2007). Also, Neretva valley is one of the most northern breeding areas of citrus in Europe, which is an important limiting factor which affects the Mediterranean fruit fly during the winter months. In order to understand the benefits of the application of the SIT it's important to mention the presence of several ornithological and ichthyological natural reserves and protected sites in the surrounding area of growing mandarins in the Neretva valley. This favor the usage of ecological methods of pest control. Bearing in mind that the Mediterranean fruit fly attacks when fruits are mature, the farmers are limited to the application of pesticides during that period of fruit development . The EU market is very rigorous and sensitive to the presence of residues of pesticides and some importing countries like Serbia or Russia (which represents a significant and constantly growing export market), put the Mediterranean fruit fly on the A1 quarantine pest list, which represents a very demanding set of conditions to the export path of the Neretva mandarins (Bjeliš et.al. 2008, Bjeliš et.al. 2010).

Due to a number of reasons presented, it was decided to apply a world-wide tested method of combating this pest, the sterile insect release method for pest suppression, which represents a non-pesticide, environmental friendly and selective method that involves laboratory mass rearing of a large number of mediterranean fruit fly sterile males, which are then released into the nature and orchards (Hendrichs et. al. 2002.). The sterile males are released into nature in large quantities twice a week , where they compete with males from natural "wild" populations. The continuous matings between sterile males and wild females result in the reduction of the offspring, contributing for less larvae in the fruits.

In order to properly carry out the implementation of the sterile insect technique SIT method, the Ministry of Agriculture, Fisheries and Rural Development has provided capital investment for the construction and equipment of the fly emergence and release facility for the implementation of the SIT programme, with the important cooperation of the International Atomic Energy Agency (IAEA) through the Technical Cooperation Programme.

Material and methods

Construction and equipping a sterile fly emergence and release facility

A facility for the implementation of the SIT programme was built in 2010. It is located in city of Opuzen, county Dubrovačko - Neretvanska and occupies an area of 180 m². The facility, in its full capacity, enables the development of 30 million sterile pupae per week, which meets the needs of the program for the entire fruit growing area of the valley. The funds are not yet sufficient to treat the whole area, so the program now operates and covers a total of 4000 ha, where the majority of the mandarin plantations are located.

The facility is organized into several rooms with the purpose of ensuring an adequate management of the sterile flies and consists of:

1. Reception and packing room for sterile medfly pupaes: controlled laboratory conditions, equipped with volumetric automatic packing line and paper bag sealing machine;
2. Laboratory for quality control of the sterile pupaes: controlled laboratory conditions, equipped with pupal counter machine, precision balance, plexiglasse cages for adult holding, freezer etc.
3. Food preparation room: equipped with a device for mixing the finished adult food, balance, equipment for agar food preparation
4. Two separate holding rooms, aproximately 30 m² each for the emergence and development of sterile males: controlled laboratory conditions, humidifiers and dehumidifiers, day – night conditions, air changing equipment, trays for paper bags, towers type Mubarqui.
5. Chilling room for the cold treatment and collection of the flies prior to its release with ground release machines in the field: chilling device, collection table;
6. Dark room: laboratory for the discrimination of sterile and wild flies and identification of the fruit flies captured in traps: UV lamps, fluorescent binocutar;
7. Fruit sampling room for the development of collected fruits of Medfly hosts and examination of samples of fruit of export shipments: controlled laboratory conditions, drying device, collection tables and pots,
8. Office with computers used for data entry and the archive of reports and collected data.

Apart from the above mentioned infrastructure, for the outdoor field operations the programme uses four vehicles of which 3 are pick-up models, 3 cold machines for the release of sterile flies and 2 boats for the coverage of areas only accesible by water channels.

SIT treated area from 2010 to 2013

The suppression of the Mediterranean fruit fly began as a pilot project in 2010, with the construction of the facility for the management of sterile flies. The treated area covered only a semi-isolated mandarin production area of about 1000 ha (Bjeliš et.al., 2010, Bjeliš et.al. 2013c). This area lis ocated in the southern part of the valley, between Mala Neretva river to the north, bordered by a mountain chain to the south and east and the sea to the west. In order to prevent invasion from the surrounding areas, certain control measures were carried

out in 10 villages that were defined as buffer areas. During the year 2011 the treated area expanded to an additional 50 ha of city of Opuzen with settlements along the coast of Mala Neretva River and 200 ha of plantations near Opuzen for a total area of 1250 hectares to be treated with sterile insects. During 2012 the area under treatment has further increased to the northern border of the valley with mandarin growing for an ultimately area of 4000 ha where the majority of mandarin and other fruits in the Neretva Valley plantations are located. In the current year 2013, the same area is being treated.

Prerelease operations

Sterilized pupae arrive in the facility in Opuzen in the amount up to 14 million per week in two separate shipments during the period from mid April to the end of November. During 2013/14, the amount reach a total of 350 million sterile males, which had to be pack, mature and release in the field. Sterile pupaes are supplied from Israel and Spain mass rearing facilities. Shipment of pupae in transit must meet certain requirements. The box which contains the pupae packed into small plastic bags has to be made of firm material in order not to get damaged during manipulation at airports. Also, it should meet the isolation conditions to maintain optimal temperature so it's often made of polystyrene, clearly labeled that " it contains living organisms, handled carefully", as well as the recommended storage temperature during transportation, which must be within 15-20 °C. To achieve this temperature, it is obligatory to put artificial gel-ice and a data logger–USB stick inside the consignment in order to measure and store information on temperatures during shipment transport (Bjeliš et.al. 2013c).

Taking into consideration two fly release methods, chilled flies release and paper bag releases methods, two packaging methods are then used. For the fly releases on locations and terrains that are hardly accesssible to vechiles, flies are released from paper bags, where bags are containing 3-5000 pupae each. For the release over fruit commercial orchards and urban areas with good accesibility, the Mubarqui towers with accessories packing and holding system is used. These towers are actually cages for emergence of sterile flies. Towers consist of 16 levels each with a maximum capacity of 50.000 pupae per level or 800.000 pupae per tower. Before packing, sterile pupes are placed into a pupal counting machine in order to measure the volume of flies for volumetric packing. These data are relevant for the next phase, which is dosing the pupae using a semi-automated volumetric filling machine with a pre-made range of volumetric units in order to select the one that responds to the given volumetric value. The food used in paper bags is dry adult food, Mubarqui type consisting of sugar and proteins, but with no water so it is mandatory to add water in the holding room after flies start to emerge inside bags. Water can be taken by the flies through the paper permeable material. For Mubarqui tower system, dry food based on sugar and proteins is used as well, but with addition of agar cakes that contains both food and water and also pads soaked with additional water. Each tower level is equipped with an additional plastic material in zig-zag shape to increase the surface and ensure adequate space for the pupae preventing its damage.

After the packing, paper bags and towers containing sterile pupaes are placed into holding rooms, in climatised and controlled conditions that ensured 21 to 25˚C and 55- 65 % relative humidity. A mechanism for humidification that controls the relative humidity in the air, a dehumidification device that annulates external influences by collecting and acumulating extra humidity and a lighting system called "day-night" that simulates natural conditions of daylight duration. A ventilation system is also built in which enables the exchange of the complete air volume in the rooms 1-6 times per hour in order to eliminate CO_2. Produced during sterile flies maturation. A day or two after packing, flies begin to

emerge from pupae and after maturation in controlled conditions, the flies are sexually mature and full of energy, for release. The fruit flies are held in holding rooms aproximately for 6-7 days, after which are released in nature. In order to improve the sexual performance of the sterile males and encourage their sexual acivity in the fiels, 24 hours before the release they are exposed to aromatherapy ventilated air with 0,3 ml/m² of ginger root oil (Shelly et.al.2007.)During the proces of sterile flies reception, packing, maturation and after chilling, quality control tests are running routineously on a weekly base (Calkins and Parker, 2005). After sterile pupaes reception in the facility, the boxes are open and temperature of the packed pupae is checked. After weighing of each bag containing the pupae and checking indicators of sterility, the status of hypoxia in bags with pupae is checked. An average sample is taken to the laboratory to run the routine standard quality control (QC) tests and to obtain information on the average weight of the pupae. The routine QC tests carried out in Opuzen facility are: emergence after 48 and 72 hours after packing and at the day of release, flight ability tests in black tubes, stress test, tests to measure the impact of the cold treatment based on the percentage of flyers after chilling. Furthermore, the total amount of collected flies is measured in kilos and the average weight of one adult fly in order to calculate release rate. In addition to these tests, field cage tests are rune in order to check matting competitiveness of sterile males compared to the wild flies (Bjeliš et.al. 2013c).

Release operations

The release of sterile males from paper bags is used on small parcels intersected with numerous water channels and terrains not accessible to vechiles. Release of paper bags covers 45% of the entire treated area using in average 1.300 paper bags with 3-5 million of sterile flies per week. The release in the field is conducted according to 10 predefined release routes. Paper bags are opened in each release point by walking in the lines inside fields in order to get good coverage and flies dispersal with goal to obtain an average density of 1500 flying flies per hectare. Important but innaccesible for vehicles areas of the valley are treated using two boats releasing the flies aside the water channels where the fruit orchards are present.

The sterile males that are packed in the Mubarqui towers are released using the ground release machines mounted on pick up vehicles. Once the flies are ready for release, they are chilled in chilling rooms, collected and transferred to the ground release machines. The cold treatment is conducted in a chilling room equipped with a cooling system which in exactly a given time the temperature reaches the required 1°C. On this temperature flies are being shocked which makes them immobile. Once the flies are chilled, all accessories from the each level of Mubarqui towers system (the food containers, pads and plastics) are taken out from each level and flies are set in collection table. Up to 6 million sterile fly adults are being collected in this manner on a weekly basis. Thus chilled adult flies are transferred to the releasing machine, which also were precooled at 2-3 °C.

The program is using 3 newly adopted ground release machines with capacity of up to 3 million of sterile child flies each (Bjeliš & Popović, 2012), that were improved from the first prototype built in 2011. (Bjeliš et. al. 2013a). The devices are fixed on iron constructions so they can be placed and displaced from the official pick-up vehicles as needed. The releasing device contains a cooler system with aluminum container where the 2-5 °C temperature is maintained during the release time in order to flies can be correctly managed for its mechanical release. The system is driven by two sources of energy: gasoline engine that runs the cooler and the blower and a recording device that connects to the 12 V power inside the vehicle. The driver manages all operations from the vehicle by using a control box to switch on or off the ventilator, and also to determine the rotational speed of the worm gear that

regulates the speed and quantity of sterile flies expelled considering the car speed. The releases in the field are carried out based on pre-determined routes taken by GPS system. By this release method approximately 55% of total area is treated.

Trapping system

In order to monitor the occurrence and captures of the Mediterranean fruit fly, and to discover new hot spots throughout the Neretva valley, a trapping network is set of approximately 140 traps type Tephri traps, in the treated and the untreated area (Bjeliš et.al. 2013c). All traps uses 3 component lures: trimethylamine, ammonium acetate and putrescine with the addition of a piece of insecticide DDVP. Beside use of this trapping system for the detection of outbreaks and wild medflies population fluctuation during season, this trapping system is a very good tool to compare the ratio of sterile and wild males in the field. All traps are being georeferenced, identified and controlled on a weekly basis. The content of the traps are transferred to the laboratory for discrimination under the black light. Since the sterile pupaes are treated with fluorescent powder before the shipping, when examined in the laboratory for identification under UV lamps, sterile males are clearly distinguished from the wild ones. With this method, the dispersion of the sterile flies in the treated area is weekly evaluated. Trapping datas are presented as flies per trap per day (FTD) and ratio between sterile males and wild males is compared.

Fruit infestation

In order to determine and evaluate the effect of the sterile insect technique (SIT) method and calculate the efficiency of reducing damage of the Mediterranean fruit fly, primary medfly host fruits are being sampled. Maps with host orchards distribution are developed with information on the precise location of parcels of the major medfly hosts in the valley such as mandarins, *Citrus reticulate*, apricots, *Prunus armeniaca*, plum, *Prunus domestica*, peach and nectarine, *Prunus persica*, fig, *Ficus carica*, persimon, *Diospyros kaki* etc. with their maturation time. Collected fruits are transferred to the fruit sampling room, counted, weight and placed on the tables with mesh in order to collect all medfly larvae's. Larvae collection is recorded every day during 3 weeks after placement.With this aim, over 2 tons of different medfly host fruits were collected every season during the maturation period to evaluate the infestation and calculate effectiveness of SIT. Fruit infestation is expressed as number of larvae/kg fruits and number of larvae/ fruit. With such data's, is possible to calculate effectiveness of the suppression method between treated and infested area using formula developed by Abbot. This formula is recommended to use when datas are available for infestation or live individuals for uniform population.

Efficacy by Abbot (%) = 100 x (mc - mt) / mc, where *mc* is mean number of live insects in treated and *mt* is mean number of live insects in no treated. (Abbott, 1925)

Sanitation

In order to detect the first infestation produced by overwintering generation of medfly, huge number of small fruit samples are collected every week following host fruits maturation, started with apricots, *Prunus armeniaca* and peaches, *Prunus persica* . This samples are bring to the laboratory and inspected in order to find medfly larvae. When larvae's are detected in specific orchard or in several orchards of same crop a systematic sanitation process on a weekly base is undertaken and all fallen fruits and fruits remain on the trees are collected and destroyed. Sanitation is focused on the stone fruits, mainly apricots and peaches and is

organized on the way that all stone fruits orchards are mapped and sanitation is carried out from the beginning to the end of picking time.

Control of export shipments

In addition to the above procedures with the fruits from the field, the rate of the presence of the medfly larvae's in the export shipments of mandarin is carried out. Approximately 40 samples per season are taken from the export shipments. The sample consists of 200 fruits that are individually placed on the development process and the infection appearance is monitored. All these operations are carried out in the fruit sampling room, in a controlled environment with the aim of accelerating the development of the larvae in the fruit of the host in order to get precise information in relatively short period of time (Bjeliš et.al. 2013b). The fruits from the samples are placed on tables or individually in containers for development where the larvae, after the development process finishes, leave the fruit or drop down through the wire.

Results and discussion

Two main parameters to measure the performance of the sterile insect technique in the field and suppression efficacy that was used in Croatian project in Neretva river valley are fruit infestation in main medfly hosts and ratio between sterile males and wild males. Even there are several important fruit crops, evaluation of fruit infestation were done on peach, *Prunus persica* L., fig, *Ficus carica* L. and mandarins, *Citrus reticulate* B. The technical index called the "sterile to wild medfly male's ratio" indicates the level of sterile males competing with the wild males for copulation with wild females in the field.

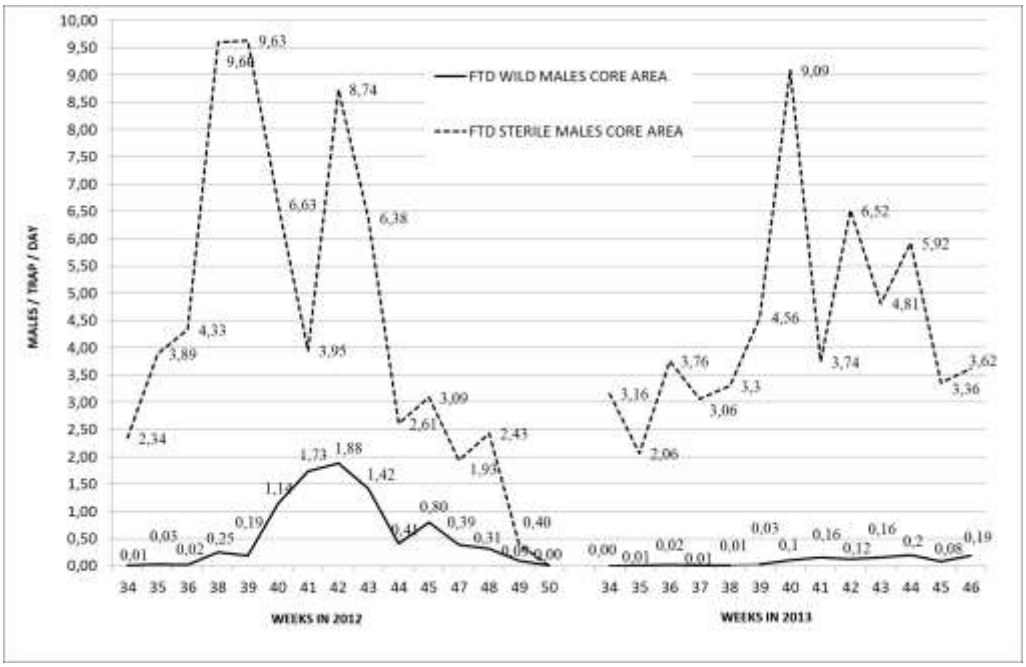

Figure 1. Ratio between sterile and wild medfly males in area where sterile insect technique was applied during 2012-2013.

This ratio is given using the capture index "Flies per Trap per Day" (FTD) which is obtained from the weekly trapping applied in the SIT treated area. In Figure 1 the sterile to wild

Medfly males ratio obtained during 2012 and 2013 is shown, being the basic principle for the effective control of the pest by this technique.
An effective and practical way to evaluate the effectiveness of the SIT is comparing infestation levels in neighboring SIT Treated and Non-Treated areas. Fruit of the main fruit fly hosts are collected in high numbers and under the same conditions and dates in both areas. The fruits were placed in special tables for maturation and collection of all the larvae coming out from the fruits for pupation. At the end of the maturation and collection of larvae outside falling in boxes, the fruit is finally dissected to collect the residual larvae inside. By counting the medfly larvae from both areas, the infestation level is obtained and given in the form of "larva per kilo of fruits collected and sampled". In Figures 2 and 3 the results of the evaluation are given based on sampling of peach, fig and mandarin as the main host fruits protected in this programme, for 2012 and 2013 respectivelly.

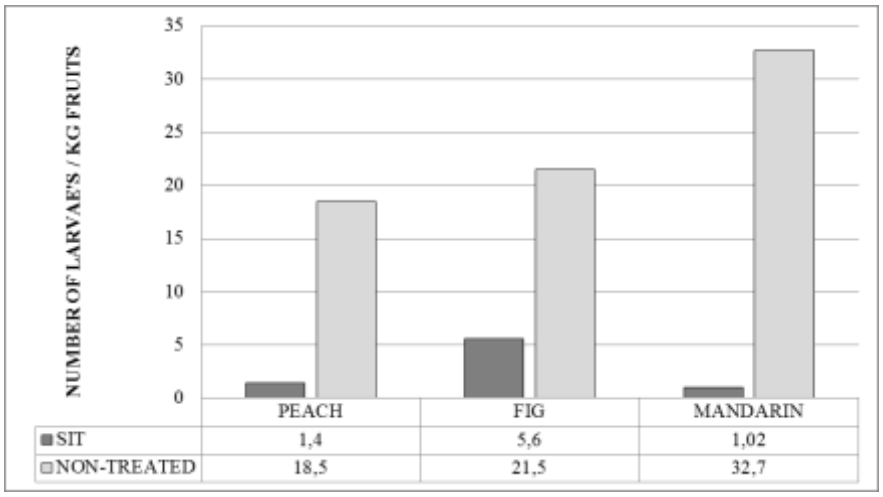

Figure 2. Fruit infestation in SIT treated and non-treated area in Neretva valley during 2012.

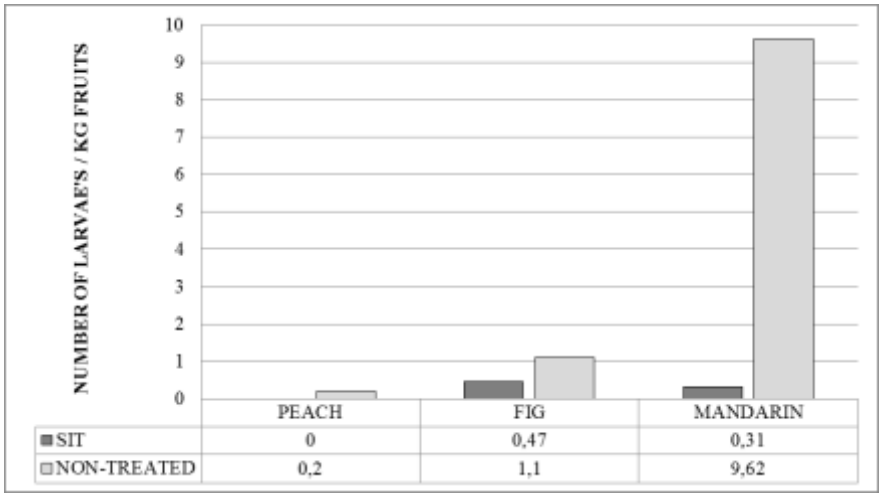

Figure 3. Fruit infestation in SIT treated and non-treated area in Neretva valley during 2013.

As the export of mandarins from the Neretva valley is the main economic activity of this region, and the Medfly is a limiting factor to increase this activity, a special evaluation was carried out by the sampling of fruits per export shipment in order to know the benefits of the SIT by reducing the percent of infested shipments and percent of the infestation inside shipments, and provide better conditions for the admissibility of the mandarins in strict markets.

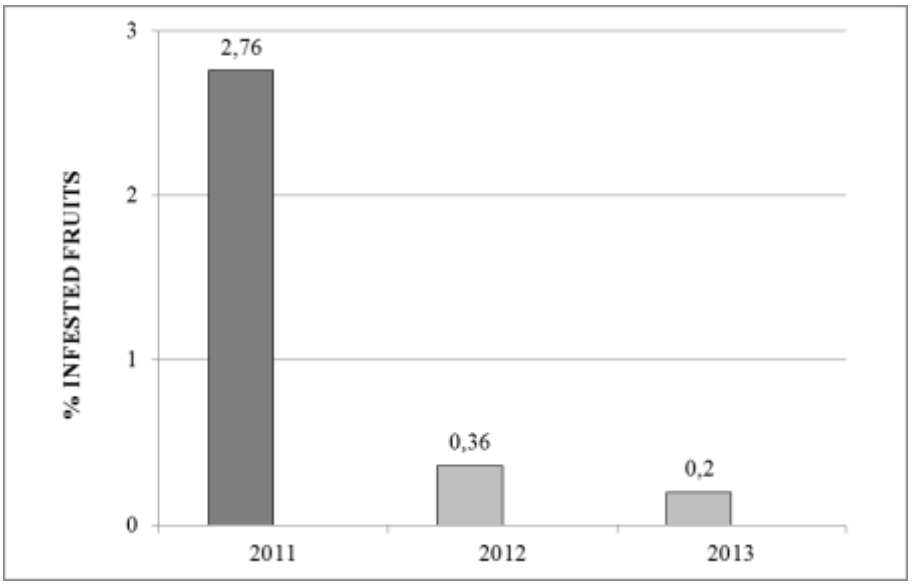

Figure 4. Average infestation of mandarin's fruits by medfly larvae's in export shipments before (2011) and after (2012 and 2103) expanding SIT treated area.

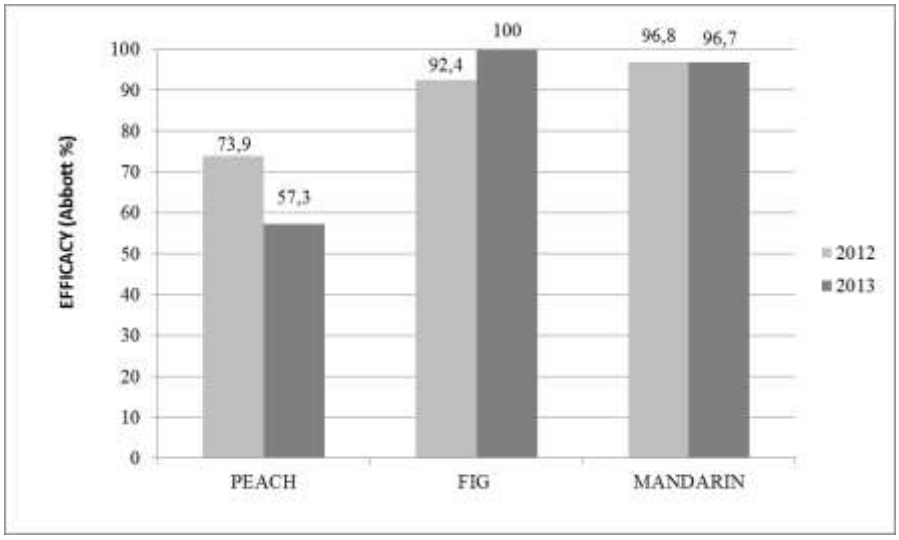

Figure 5. Efficacy of the Sterile Insect Technique for suppression of Medfly in Neretva valley of Croatia.

In Figure 4, the results of this special sampling are given for 2011 (before expanding program), 2012 and 2013, observing the significant reduction of infested shipments from year to year. Furthermore, it is important to stress that from previously almost 100% of shipments containing infested fruits, after expanding of area, the percentage of infested shipments were reduced to 53,3% in 2012 and 26% in 2013.
The efficacy of suppression of Medfly by sterile insect technique is shown in Figure 5. Efficacy was evaluated based on the comparisons of infestation level measured by number of larvae per kilo of fruits as shown in the Figure 2 and Figure 3 for three most important crops (peach, fig and mandarin). The infestation level between sterile males treated area compared to the north part of the valley where control methods did not conduct was used to calculate efficacy. The results of the application of the Sterile Insect Technique as a species selective and environmentally acceptable method of combating the Mediterranean fruit fly *Ceratitis capitata* Wiedemann over an area of 4000 ha, confirms the high effectiveness of the SIT for the suppression of Mediterranean fruit fly in the ecological conditions of the Neretva valley. Based on the obtained results, it is to expect that this method will be applied to the entire area of the Neretva valley including the side of Croatia and Bosnia and Herzegovina as an area-wide approach.

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References

Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol.; 18 : 265-267.

Bjeliš, M. 2004. Potential for medfly, Ceratitis capitata Wied. (Diptera, Tephritidae) control in Croatia. Proceedings of the International Socciety of Citriculture, Volume III, 10th International Citrus Congress, Agadir, Maroco, pp.953-959.

Bjeliš, M. & Pelicarić, V. 2004, Fruit flies in Croatia; Owerview of damage and current controll strategies. In: Barnes, B. (ed.), Proccedings of VIth International Symposium of Fruit Flies of Ecconomic Significance, Stellenbosch, South Africa, May 2002. pp. 325-329.

Bjeliš, M. 2007. Feasibility study of medfly (Ceratitis capitata Wied) control by sterile insect technique in Neretva river valley. Lectures and papers presented at the 8th Slovenian conference on plant protection, Radenci, March 6-7.193-198.

Bjeliš, M., Radunić, D., Masten, T., Kotlar, A. 2007. Spatial distribution and temporal outbreaks of medfly Ceratitis capitata Wied. (Diptera, Tephritidae) in Republic of Croatia. Lectures and papers presented at the 8th Slovenian conference on plant protection, Radenci, March 6-7. 321-325.

Bjeliš, M., Ljubetić, V., Novosel, N. 2008. Control of medfly by SIT in the Neretva river valley. Fruit flies of Economic Importance: From basic to applied knowledge. Proceedings of the 7th International Symposium on Fruit Flies of Economic

Importance, R.L.Sugayama, R.Zucchi, S.M.Ovruski, J.Sivinski (eds), Biofabrica Moscamed Brasil, Salvador, 2008, Brasil, pp. 255-259.

Bjeliš M., Marušić I., Popović L. 2010. SIT Pilot project in Croatia: Control of medfly by SIT in the Neretva river valley, 8th International Symposium on Fruit Flies of Economic Importance, Valencija, Španjolska, Proceedings of the 8th International Symposium on Fruit Flies of Economic Importance. B.Sabater Munoz, V.Navarro Llopis, AUrbaneja Garcia (eds), Editorial Universitat Politecnica de Valencia, Valencia 2010. pp.200-205.

Bjeliš, M., Popović, L. 2012. Use of the ground release machines in neretva SIT program for the release of the high quality chilled sterile males. TEAM 2nd International Meeting "Biological invasions of tephritidae:ecological and economic impacts", Kolymbari, Greece, Abstracts, pp.138.

Bjeliš, M., Radunić, D. and Bulić, P. 2013a. Pre- and post-release quality of sterile Ceratitis capitata males released by an improved automated ground release machine. Journal of Applied Entomology, 137 (Suppl.1), 154-162.

Bjeliš, M. Popović,L. Deak, S., R.Cardoso-Pereira 2013b. Control procedures for presence of Mediterranean fruit fly in export shipments of mandarins from Neretva during 2011-2012. godine. 8th Scientiffic meeting of Croatian fruit growers, Zagreb, 1-2. March.2013. Abstracts, pp. 112-113Zbornik sažetaka,pp.112-11

Bjeliš, M., Popović, L., Deak, S., Buljubašić, I., Ivanović, A., Arnaut, P., Pereira, R. 2013c. Supression of mediterranean fruit fly by SIT over 4000 ha of fruit orchards in Neretva river valley. Lectures and papers presentd at the 11th Slovenian conference of plant protection, Bled, March 5-6th 2013. / Trdan, Stanislav (ed.). - Ljubljana: Plant Protection Society of Slovenia, 2013, pp. 101-108.

Calknins, C.O. & Parker, A.G., 2005. Sterile insect quality. In: Sterile Insect Technique. Principles and Practice in Area-Wide Integrated Pest Management, Springer Link, Editors: V. A. Dyck, J. Hendrichs, A.S. Robinson pp 269-296.

Hendrichs, J., Robinskon, A.S., Cayol, J.P., Enkerlin, W.R., 2002. Medfly area wide sterile insect technique programmes for prevention, supression or eradication: the importance of metting behaviour studies. Florida Entomologist 85, 1-13.

Pelicarić, V., Bjeliš, M., Kotlar, A., Kirigjija, I., Caput, I. 2001. Mediterranean fruit fly, *Ceratitis capitata* Wied. (Diptera, Tephritidae) – Pest of growing importance in Dalmatia. Abstract from 45th meeting of Croatian plant protection society, Opatija, 13-16. February,2001. No.1, pp. 29-30. (in Croatian).

Pelicarić, V. & Bjeliš, M. 2002. Results of detection and monitoring of Mediterranean fruit fly, *Ceratitis capitata* Wied. (Diptera, Tephritidae) during 2001. Abstract from 45th meeting of Croatian plant protection society, Opatija, 12-15. February 2002. No. 1, pp.15-16. (in Croatian).

Shelly, T., McInnis, D.O., Rodd, C., Edu, J., Pahio, E., 2007. Sterile Insect Technique and Mediterranean Fruit Fly (Diptera: Tephritidae): Assessing the Utility of Aromatherapy in a Hawaiian Coffee Field. Journal of Economic Entomology 100(2), pp.273-282.