

SUPPRESSION OF MEDITERRANEAN FRUIT FLY BY SIT OVER THE 4000 HA OF FRUIT ORCHARDS IN NERETVA RIVER VALLEY

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ABSTRACT

101 Medfly - *Ceratitis capitata* (Wied.) is a pest of high economic importance in Neretva valley, affecting production of several fruit species, specially mandarins, *Citrus reticulata* grown mainly for export to EU, former Yugoslavia countries and Russia. The export reach up to 75% of total yield valued to over 20 million of euros per year. Medfly infestation in mandarin's fruits is estimated to be 10-30% without any control measures and also causes major problems to exports due to quarantine restrictions and pesticide residues on fruits. After two years (2010-2011) of successfully suppression trough an Sterile Insect Technique (SIT) pilot project integrated with other suppression methods, conducted with cooperation of the FAO/IAEA, the Croatian Ministry of Agriculture expand the project to the whole lower part of the Neretva valley in 2012, covering over 4000 hectares of fruit orchards, mainly mandarines. For this reason, a fly emergence and release facility in Opuzen with capacity of handling 20 million of sterile flies/week was additionally equipped. Releases are performed mainly with two ground release machines, using chilled flies, mounted on vehicles. Trapping system is set and geo-referenced over the whole SIT treated and non-treated area of the valley (additional 4000 ha) with 3C lures Tephri Traps. Captured flies are checked by fluorescent lamps to separate sterile from the wild flies and provide information on the insect population level. Routine fruit sampling is undertaken to evaluate fruit infestation and suppression efficacy. Results of the two years of pilot project showed that medfly population, measured as number of larvae per kg of fruit, was reduced annually from 75,9 – 93,2% in figs and 75,9 – 99,2% in mandarins in SIT treated area compared with non-treated area. Results of the 3rd year of the project showed high level of the suppression medfly population, measured as number of larvae per kg of fruit for 73,9% in figs, 92,4% in peaches and 96,8% in mandarins.

Key words: sterile flies, mandarins, *Ceratitis capitata*, Sterile Insect Technique

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1 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, but its negative effects are manifested mainly in the export oriented production of *Citrus reticulata* B, mandarin. The Mediterranean fruit fly in the Neretva valley was first detected in 2001 (Pelicari *et al.*, 2001; Pelicari and Bjeliš, 2002; Bjeliš and Pelicari, 2004). It was estimated that the damage this pest causes in mandarin growing, in orchards as well as the economic damage observed in the export activities reach to the total of 10-30% yield, depending on the year, incidence and intensity of this pest (Bjeliš *et al.*, 2007).

The Neretva valley extends to 15 000 hectares of Croatian territory, of which 7 000 hectares are agricultural areas, where grown mandarins represent a culture of highest economic importance. 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, depending on the year, amounts 40 – 60.000 tons and increases each year due to many young plantations, with estimated annual production of 100.000 tons in a few years. In order to undertake and implement legal measures for suppression of the Mediterranean fruit fly, various analysis were conducted and, as a result, the most efficient measures of control were adopted. Studies of economic and technical feasibility recognized SIT (Sterile Insect Technique) as the best method for suppression of the Mediterranean fruit fly in the Neretva valley for several reasons. 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 a limiting factor for the effect of the Mediterranean fruit fly during the winter months. In order to understand the benefits of this method it's important to mention the presence of several ornithological and ichthyological reservations and protected sites in the area of growing mandarins in the Neretva valley, in favor of the usage of ecological methods of suppression. Bearing in mind that the Mediterranean fruit fly attacks when fruits are mature, the farmers are limited to the application of pesticides. 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 mandarin (Bjeliš *et al.*, 2008, 2010).

Due to a number of reasons presented, it was decided to apply a world-wide tested method of combating this pest, a sterile insect method of suppression, which represents a non-pesticide, environmentally friendly and selective method that involves laboratory mass rearing of a large number of sterile males, which are then released into nature and orchards. These males are released into nature in large quantities, where they compete with males from natural, "wild" populations. Sterile males do not give offsprings in copulation with fertile females from the wild population so the damage can't be manifested by the presence of larvae in the host fruit. In order to properly carry out the implementation of SIT method, through cooperation of the International Atomic Energy Agency (IAEA) and the Ministry of Agriculture, Fisheries and Rural Development, through the Croatian Technical Cooperation Project TCP RER 5014, with capital investment of the Ministry, the necessary funds were provided for construction (2010) and equipment of facilities for the implementation of SIT programs.

2 CONSTRUCTION AND EQUIPPING OF STERILE FLIES PACKING AND EMERGENCY FACILITY

Facility for the purpose of implementing the SIT program was built in 2010., it is located in city of Opuzen, county Dubrova ko - neretvanska and occupies an area of 180 m². The facility, in its full capacity, enables the development of 20 million sterile pupae specimens per week, which meets the needs of the program implementation in the entire 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 productive mandarin plantations is located.

The facility is organized into several rooms, with a purpose of ensuring the adequate implementation of the program and consists of:

1. Room for reception and packing of sterile pupae of the Mediterranean fruit fly: controlled laboratory conditions, equipped with volumetric automatic packing line, paper bag sealing machine;
2. Laboratory for quality control of the sterile pupae shipments: controlled laboratory conditions, equipped with pupal counter machine, precision balance, plexiglas cages for adult holding;
3. Room for food preparation and drying: equipped with device for mixing of the food, balance, equipment for preparing and cooking food from agar;
4. Two separate holding rooms for the emergence and development of sterile males: controlled laboratory conditions, humidifier and dehumidifier, day – night conditions, air changes equipment;
5. Rooms for cold treatment and collecting of adult sterile insects for machine release: chilling device, collection table;
6. Laboratory for the examination and identification of the catch from traps: UV lamps, fluorescent binocular;
7. Rooms for the development of collected fruits of hosts and examination 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 structure of the facility for SIT technique, for the outdoor field operations, the program uses four SUVs of which two pick-up models, two devices for machine release of sterile individuals and two boats for a full coverage of hardly accessible areas.

3 TREATED AREA FROM 2010. TO 2103.

Suppression of the Mediterranean fruit flies began as a pilot project in 2010., as well as the construction of the facility for the implementation of SIT programs. The treated area covered only a semi-isolated area called Vidrice, of about 1000 ha (Bjeliš *et al.*, 2010). Vidrice are located in the southern part of the valley, between Mala Neretva river to the north, bordered by 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 villages Lovorje, Tuštevca, Pižinovca, Otok, Mihalj and Trn, as well as in the villages Vlaka, Buk and Crepina, that defined a buffer zone. During the year 2011 treated area has been expanded to an additional 50 ha of city of Opuzen with settlements Jasenska, Trnovo and Pinovac, area along

the coast of Mala Neretva River and 200 ha of plantations near Opuzen which was in total 1,250 hectares treated with sterile insects.

During 2012. the area under treatment has further increased to the north-border area for mandarin growing, villages Šari Struga, Banja and Rogotin, whole southern-east area of the valley: Komin, the whole Jasenska, Modri , locations Aerodrom, Crepina, Glog, Uš e, Prunjak, Blato, Vidrice canal, Mala Neretva and Rastoka which ultimately represents the treatment of 4000 ha of land, better to say the majority of grown mandarin plantations and other fruits in the Neretva Valley. In the current year 2013., the same area is being treated.

4 ACTIVITIES IN THE FACILITY BEFORE RELEASE

4.1 Reception

Sterilized pupae arrive in the facility for applying SIT techniques in Opuzen in the amount of 11-14 million pupae per week, in two separate shipments, during the period from mid - April to the end of November, while the Mediterranean fruit fly is active in plantations. In the year 2013. the amount will reach a total of 350 million sterile males, packaged, bred and released. Pupae are supplied by a Spanish producer TRAGSA. Shipment of pupae in transit must meet certain requirements. The box which contains the pupae packed into small bags has to be made of firm material in order not to get damaged during package manipulation at airports. Also, it should meet the isolation conditions of temperature preserving so it's often made of polystyrene, clearly labeled and that it contains living organisms and should be handled carefully, as well as 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 about the temperature in the shipment during the transport.

4.2 Quality control during development

After receiving the consignment in the facility, the box is opened and using digital thermometers the average temperature of the shipment is measured. After weighing each bag containing pupae and checking indicators of sterility, the status of hypoxia in bags with pupae is checked, the average sample is taken to the laboratory for quality control and used as the basis for obtaining information about the average weight of pupae and the material for setting the prescribed standard of quality control tests carried out for each shipment such as: stress test-a % of surviving flies in conditions without food and water in total darkness after 48 h. Other tests performed are: obtaining data on the percentage of flyers from shipments depending on the method of packing, % of deformed specimens, emergence after 72 hours after packing and at the very day of release and tests measuring the impact of cold treatment on the percentage of flyers. Furthermore, the total amount of collected flies is measured in kg and the average weight of developed units (in mg). In addition to these tests, once a year cages are set out in the field where they simulate natural conditions so it is possible to observe compatibility of sterile males compared to the fertile ones in copulation with females from the wild population.

4.3 Packaging

After receiving the consignment and carrying out all necessary operations, begins the packaging process. Taking into consideration two ways of release, we use two methods of

packaging. For release on locations and terrains that are hardly accessible to vehicles, we use paper bags, each containing 500 pupae and towers of type Mubarqui (Mexico). These towers are actually cages for emergence of grown units, they consist of 16 levels each, with maximum capacity of 50.000 pupae per level or 800.000 pupae per tower in total. Units that are packed in towers are being used in ground release with vehicles. After receiving the pupae and taking a relevant sample, they are placed into a machine with mechanism for counting, weighed and their volume is determined. Based on the results that we get on 5000 pupae, we calculate volume of 50.000, in order to determine the volume of the pupae placed in each level of the tower. These data are relevant for the next phase, which is dosing the pupae using a semi-automated 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 packaging paper bags is so called dry food, consisting of sugar and proteins, but with no water added so it is obligatory to add water in some way on a daily basis, through the bags which are made of adequate permeable material. For packaging the towers, dry food based on sugar and proteins is used as well, but with addition of agar food that contains both food and water and pads soaked with additional water. Each tower level is equipped with an additional plastic material to increase the surface, ensure adequate arrangement of pupae in zig-zag shape, preventing damage of pupae.

4.4 Development in holding rooms

After the packaging, paper bags containing pupae and packed towers are placed into air-conditioned rooms, in controlled conditions that are ensured by air-condition (21 – 25 °C, 55-65 % relative humidity), a mechanism for humidification that controls the relative humidity in the air, a special dehumidification device that annulates external influences by collecting and accumulating extra humidity and a lighting system called „day-night“ that simulates natural conditions by duration of daylight. A ventilation system is also built in, which enables exchange of complete air volume in the rooms 1-6 times per hour. The ventilation system helps reduce CO₂, created as a by-product of the breathing process of sterile insects that can be harmful for their later performances. The fruit flies are held in these controlled conditions for 6 to 7 days, after which are released in nature. In order to improve the sexual performances of the sterile males and encourage their sex drive, 24 hours before the release, they are exposed to ventilated air with concentration of 0,3 ml/m of ginger oil.

5 RELEASE OPERATIONS

5.1 Release of paper bags

Release of paper bags is used on small parcels intersected with numerous canals and terrains that are not accessible to vehicles. In two – three day period after the packaging, flies begin to emerge from pupae and on the sixth day of development in controlled conditions, the flies are sexually mature and full of energy, which is optimal for their release. Release of paper bags covers 45% of the entire treated area and is conducted according to pre-defined routes. So far, ten release routes are defined, which is enough to cover 5 million of sterile units released weekly, in 1000 packed paper bags. A part of the above mentioned 45 %, applies to the inaccessible areas treated with two boats on three defined routes.

5.2. Releasing sterile units by semi-automatic machine

For machine-release we use mature units from second method of packaging – towers with levels. Towers are presenting cages in which flies are moving and flying. In order to open the cage and transfer flies to the releasing machine, cold treatment has to be made.

Cold treatment is conducted in a specific room equipped with cooling system which in exactly given time-term lowers the temperature on required 1°C. On this temperature, flies are being shocked which makes them immobile but it does not cause any damage. Previously set food containers, pads and plastic are taken from each level and flies from the levels are being collected by using collecting table. 6 million sterile units are being collected in this manner on a weekly basis. Thus prepared units are transferred to the releasing machine.

The program has two semiautomatic devices for releasing sterile units (Bjeliš and Popovi , 2012). The devices are fixed on iron constructions so they can be placed and displaced from official pick-up vehicles if needed. The releasing device contains a cooler with aluminum container where the 2-5 °C temperature is maintained during the release, an installed ventilator for preventing moisture condensation in the container, fly-holder, worm gear on the bottom of the container for dosing flies in the releasing pipe, blower engine which by “venturi effect” absorbs under-cooled and dosed insects in the tube and pressurized air expels flies through the tube. The system is driven by two sources of energy: gasoline engine that runs the cooler and 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 the control box and controls the switching on and off the ventilator, determines the rotational speed of worm gear that is, regulates the speed and quantity of sterile units . Releasing is carried out on the routes taken by GPS system, there are seven altogether and they cover 55% of the total treated area of 4.000 ha. These machines are prototypes of Plant Protection Institute, and as such represent an innovation in SIT programs (Bjeliš *et al.*, 2013a)

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6 METHODE OF THE EVALUATION OF EFFICASSY

6.1 Adult captures

In order to monitor the occurrence and catch of the Mediterranean fruit fly, and discover new hot spots throughout the Neretva valley, a network is set of 136 traps type Tephra Trap, 113 in the treated and 23 in the untreated area. All traps contain attractants 3FA-trimethyl-amine, ammonium acetate, putrescine, with the addition of insecticides DDP. Besides tracking natural populations to compare the effectiveness of programs and methods, the intensity of wild populations in the treated and untreated area is also observed. Traps placed in the treated area also have the purpose of monitoring sterile populations. Since the sterile units are treated with fluorescent powder before the shipment, when examined in the laboratory for identification under UV lamps, they are clearly distinguished from the wild ones. With this method the dispersion effectiveness of sterile units in the treated area is being evaluated as well as the employees in their releasing activities. All traps are being identified and controlled on a weekly basis. Results shows that FTD (Flies per trap per day) ratio between sterile and wild population in favor of sterile flies during end of August (FTD Ratio sterile/wild= 230) and during end of September (FTD Ratio sterile/wild=50,6) what is a prove of good dispersion of the sterile flies in the field.

6.2 Rate of fruits infestation

In order to determine the effect of SIT method and calculate the efficiency of reducing damage on the Mediterranean fruit fly, fruits of the host of this pest are being sampled.

Facility for the implementation of SIT technology possesses precisely made maps with marked parcels of major hosts in the valley, except citrus fruits, such as apricots (*Prunus armeniaca*), plum (*Prunus domestica*), peach and nectarine (*Prunus persica*), fig (*Ficus carica*) and japanese apple (*Diospyros kaki*) to their maturation time. These maps also serve for further treatment while releasing sterile units in these target areas. Since the whole Neretva valley covers about 7,000 hectares of agricultural land and the treated area in the year 2013 is 4000 ha, collecting samples of fruits of hosts on treated and untreated area, gives a clear picture of efficiency by repeated patterns during the season. That way, in the previous 2012, 1300 kg of fruits were collected from the ground through the period of hosts' maturation to evaluate the effectiveness of applied technique. We will list the results of reduced infection in the treated area compared to untreated area which is calculated by the number of larvae / kg which presents the index of infection. The results have showed that the applied SIT technique resulted in a decrease of the infection index in figs fruits by 75.9% and 99.2% in mandarins during the 2012.

6.3 Control of export shipments

In addition to the above procedures with the fruits from the field, the rate of the presence of the Mediterranean fruit fly in the export shipments of mandarin is carried out. A sample is taken from the export shipments and during the season 40 samples are being sampled. The sample consists of 200 fruits that are individually placed on the development process and the infection appearance is being monitored. All these operations are carried out in the fruit sampling room in the part of SIT technique facility, 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.

The fruits 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, than larvae are being collected and recorded.

After two years of implementation of the above analysis and 40 samples processed per year, the number of positive samples has been reduced from approximately 90% during the 2011, to about 50% during the 2012 (Bjeliš *et al.*, 2013b).

7 CONCLUSION

The results of the application of the Sterile Insect Technique (SIT techniques) as species selective and environmentally acceptable method of combating the Mediterranean fruit fly *Ceratitis capitata* Wied. over the 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 to the side of the Croatia and Bosnia and Herzegovina.

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