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REGIONE SICILIANA

ENTE DI SVILUPPO AGRICOLO

A BIOFACTORY IN SICILY FOR THE BIOLOGICAL ERADICATION OF THE *CERATITIS CAPITATA* IN THE MEDITERRANEAN COUNTRIES. [1]

1. INTRODUCTION

In 1996, Ente di Sviluppo Agricolo (E.S.A. - Sicilian Agency of Agricultural Development) was assigned by the Regional Government of Sicily the task to foresee the possibility to carry out some programmes related to a system of biological control in agriculture.
Since then, E.S.A. has produced:

- the required projects to accomplish a biofactory for the "*useful insects*", which will be built in Ramacca (Catania). Three species of "*useful insects"* (Aphytis melinus, Criptolaemus Montrouzerii, Leptomastix dactylopii) was provided for the biological control of Citrus fruit growning;
- a preliminary project, together with a feasible socio-economic and environmental analysis about regional activities of the system for the biological eradication of Ceratitis capitata (fruit medfly), with the co-operation of the FAO-IAEA Agriculture and Biotechnology Laboratory.

The necessity to turn to alternative technologies is due to the consideration that the fruit medfly, whose country of origin is Morocco but now it has a wide-world diffusion, is considered as the most noxious fly for the fruit-growning in the Mediterranean and sub-tropical countries.
The logical consequences of the damages caused by the fruit-medfly affect the low production, the difficult commercialisation of the following agricultural products (Citrus fruit, Peaches, Pears, Apricots, Japanese Medlars, Persimmons, Figs, Indian Figs), and such products do not reach high quality standards imposed by international markets.
As to the control of the medfly, the traditional use of chemicals create several problems such as: toxicological aspects related to the consumers and the agriculture farmers, and the impossibility to operate over large cultivated areas.
The necessity to comply with the European Community requirement about the quality of the agricultural products, without the use of chemicals, or at least with an accurate control, has brought the local Government to foresee the introduction of new productive technologies in the field of biological pest control and the use of chemicals in commercial orchards in the Mediterranean areas.
E.S.A. has, therefore, planned to create a mass rearing facility within Sicily to analyse the methods for the control and eradication of the fruit-medfly.
In view of the need to utilise a new and productive technology the Regional Government of Sicily has initiated a programme called the Sterile Insect Technique (S.I.T.), consisting of the introduction of large numbers of sterile male insects which are released into the target area by aircraft.

[1] elaborated by the co-projectist ESA Dr. Giuseppe Greco and traslated by Dr. Liliana De Caro

Initial development of SIT components was successfully tested in Italy (pilot project CNEN in the Isle of Procida in 1967), in U.S.A. (California and Hawaii), in Mexico (project Moscamed) and in Guatemala (Waimanaloo and Laguna del Pino).
The SIT has proved successful in the Isle of Okinawa (Japan) for the eradication of "Dacus cucurbitae", an insect like the fruit-fly. Over 200 million of sterile insects were released per week.
Recently the *Cochliomyia hominivorax* was eradicated from Libya using this technology with the co-operation of FAO, IAEA, IFAD, UNDP, CEE, MAF, Libyan Government and other institutions.
The programme, consisting in the genetic sexing separation of males from females from birth to the pupal stage, produced a large quantity of sterile insects to be released in wider territorial areas at lower costs.
The preliminary project of the biofactory contains a mass rearing facility for the fruit-medfly, which may be expanded. The base module of the structure, including the connected rooms for the irradiation equipment, has to garantee 85 million of sterile flies per week.
This project may be applied to several programmes of biological pest control to be used in different fields of agriculture.

2. ORIGIN AND BIOLOGY OF *CERATITIS CAPITATA*

The "Fruit-fly" or "Orange-fly" is a dipteron of the family of Tripetidi, improperly called "Medfly" because it's indigenous to the West Coast of Africa (Gulf of Guinea). From there, it has expanded towards tropical, sub-tropical and temperated countries because the young larvae were placed within the fruit. Its infestation reached the Mediterranean area (Spain, South France, Insular and Peninsular Italy, Dalmatia, Greece, European and Asian Turkey, Cyprus, Israel, Palestine, Lebanon, Syria, Algeria, Egypt, Libya, Morocco, Tunisia).

Ceratitis capitata (Medfly)
Photo by FAO/IAEA Agriculture and Biotecnology Laboratory.

The insect is noxious for many species of fruit plants. The larvae develop from the eggs and the affected fruits fall down and rot. The affected fruits speed up their maturation, assuming the look of ripe fruit.

The consociation of fruit plants and Citrus plants favours the attacks of the medfly. During the summer months the medfly attack to the fruit plants favours the subsequent attack to the early ripening Citrus plants thus increasing the number of its vital cycle. In the major part of cases the medfly stings in the Citrus fruits are sterile, whereas in the other fruits species the eggs develop from the larvae. However, this results in a great commercial damage.
Omitting the specialistic information about the vital cycle of the insect, it's necessary to specify that the plants attacked by the medfly are:

1. Citrus fruits as mandarin, clementine, tangelo and orange (Navelina and Tarocco; tardy cultivar are not usually attacked);
2. Fruit species as peaches, plums, apricots, pears, some apples, quinces, Japanese medlars, Indians figs, figs, persimmons, azaroles, avocados.

In central Italy we count 3-4 generations of medflies while in South Italy and in the Isles there are 6-7 generations for the following reasons:

- the adults of the medfly are long-lived; normally-fed insects live a minimum of 2 months in winter to a maximum of 7 months in spring-summer: this condition influences the fertility, assuring the perpetuation of the species;
- during the year, the number of insect generations is greatly influenced by the environmental conditions (state of maturation of host, vegetative condition according to the weather, the graduality of the maturations of the fruits, density of the cultivar, soil characteristics).

Size of the Medfy
Photo by FAO/IAEA Agriculture and Biotecnology Laboratory.

In the South of Italy (Campania, Calabria, Sicilia) the adult insect can be found from January to March-April in mandarins and oranges (first generation); in May-June in the early peaches and apricots (second generation); in July in apricots, in peaches and in some early pears (third generation); in August in badly affected peaches and pears (fourth generation); in September once again in peaches, in figs, in persimmons, in Indian figs, in Citrus fruit the ovipositing takes place, but aborts (fifth generation); in October-November in tardive peaches, in persimmons, in Indian figs, in oranges and in mandarins (sixth generation); in November-December in Citrus fruits if the season has a mild weather (seventh generation).

3. THE POTENTIAL REQUEST OF THE S.I.T. - *CERATITIS CAPITATA*

The territories of the Mediterranean Countries, cultivated with Citrus and fruit plants, presently require a biological control system of the medfly.
It's not possible to cover an enlarged area, outside the Mediterranean area, because the cost of activities related to SIT-medfly is less competitive with respect to chemical treatment for territories far from a possible biofactory, located in Sicily.
Only the evident success of SIT or the introduction of the new local laws for biological productions could bring about an increase of the market use.
The potential Mediterranean request of the SIT-medfly refers to those specific damaged areas of about 211 thousand hectares, 34% of which in Sicily, 23% in other Italian Regions and the remaining 43% in non Italian Countries of the Mediterranean area.
The above-mentioned area of 211 thousand hectares is equal to about 7,5 million topographic hectares: in fact the biological pest eradication will have to be effected by means of aircraft, covering also the free zones that are not-damaged territories.

4. THE POTENTIAL MARKET USE IN THE COUNTRIES OF THE MEDITERRANEAN AREA

This area consists of:

- 90 thousand hectares including Morocco, Northern African Countries, Spain, Southern France, Greece, Turkey, Middle East;
- 48 thousand hectares including the Region of the Southern Italy (Calabria, Campania), 70% of which is related to Citrus fruits;
- 74 thousand hectares including Sicily equal to 60% of the total area cultivated with Citrus and fruit plants, 80% of which of early oranges (Naveline, Washington Navel, Moro and Tarocco) and mandarins.

5. THE PRELIMINARY PROJECT OF THE SICILIAN BIOFACTORY. DIMENSIONAL FACTORS THAT HAVE BEEN CONSIDERED.

The planned productive level of the Sicilian biofactory amounts to 185 million of sterile medfly per week according to the following considerations.
In the presence of precise, but not-representative monitoring data related to the total Sicilian territory, some hypothetical models (which you can see in table A) have been proposed.
For each level infestation, table A shows the number of traps for hectare, the estimated female population (number for hectare), the ratio sterile-fertile males in field, to calculate to effectual release rates, expressed in weekly sterile males.

Tab. A - An hypothetical model for calculating of the release rates related to each infestation level. MTD (medfly/trap/day)

Infestation level (MTD)	Traps (n°/Ha)	Estimated Female Population (n°/Ha)	S:F Ratio	Release rate (n°/Ha/week)
10,0 - 20,0	0,12	3.750	50	3.750
5,0 - 10,0	0,12	1.875	50	1.875
1,0 - 5,0	0,05	1.875	50	938
0,4 - 1,0	0,05	2.813	50	188
0 - 0,4	0,02	4.500	50	75
0	0,02		50	75

Table 1 shows an hypothetical distribution of the areas for each infestation level (MTD or Medfly Trap Day), within each of the 5 zones.

Table 1 - The hypothetical division of Sicily into 5 zones and within each zone the probable area for each hypothetical infestation level. In this scenario does not include chemical control. (MTD = medfly trap day; Ha = hectare)

Infestation level (MTD)	Area					
	Zone 1 (Ha)	Zone 2 (Ha)	Zone 3 (Ha)	Zone 4 (Ha)	Zone 5 (Ha)	Total (Ha)
10,0 - 20,0	5.000	5.000	-	-	-	10.000
5,0 - 10,0	5.000	5.000	5.000	-	-	15.000
1,0 - 5,0	10.000	10.000	10.000	5.000	-	35.000
0,4 - 1,0	75.000	50.000	25.000	10.000	5.000	165.000
0 - 0,4	300.000	100.000	100.000	75.000	100.000	675.000
0	360.000	430.000	360.000	310.000	140.000	1.600.000
Total Area (Ha)	**755.000**	**600.000**	**500.000**	**400.000**	**245.000**	**2.500.000**

Table 2 shows that, in the absence of chemical integration, 513 million per week of sterile males will be required by years 9 and 10, equal to 7 base modules of the biofactory.

Table 2 - A hypothetical analysis of a year by year sterile fly treatment of Sicily (without chemicals), based upon 5 zones with infestation attributes outlined in table 1. The number of sterile males required for each zone and then the subsequent protection of each zone gives the total males required for protection and treatment.

Zone	Programme Year	Area (Ha)	Males request for each zone (mill/week)	Males required for protection of free zones (mill/week)	Total males required whit protection and treatment (mill/week)
1	Years 1-2	755.000	101		101
2	Years 3-4	600.000	87	57	158
3	Years 5-6	500.000	58	45	260
4	Years 7-8	400.000	36	38	400
5	Years 9-10	245.000	20	30	513

In the hypothesis of tables 3 and 4 it's important to say that, in the presence of baiting (that is with integration of chemicals) which bring back the infestation under the critic level of 1,0 MTD, a production of 185 million per week of sterile males will result very satisfactory for the eradication of medfly in about 10 years in Sicily.

Table 3 - The hypothetical division of Sicily into 5 zones and within each zone the probable area for each hypothetical infestation level after integration of chemical (baiting). The number of dense infestations is < 1,0 MTD. (MTD = medfly trap day; Ha = hectare)

Infestation level (MTD)	Area					
	Zone 1 (Ha)	Zone 2 (Ha)	Zone 3 (Ha)	Zone 4 (Ha)	Zone 5 (Ha)	Total (Ha)
10,0 - 20,0	-	-	-	-	-	-
5,0 - 10,0	-	-	-	-	-	-
1,0 - 5,0	-	-	-	-	-	-
0,4 - 1,0	50.000	5.000	5.000	5.000	5.000	70.000
0 - 0,4	250.000	150.000	150.000	100.000	100.000	750.000
0	455.000	445.000	345.000	295.000	140.000	1.680.000
Total Area (Ha)	**755.000**	**600.000**	**500.000**	**400.000**	**245.000**	**2.500.000**

Table 4 - A hypothetical analysis of a year by year sterile fly treatment of Sicily (with baiting), based upon 5 zones with infestation attributes outlined in table 3. The number of sterile males required for each zone and then the subsequent protection of each zone gives the total males required for protection and treatment.

Zone	Programme Year	Area (Ha)	Males request for each zone (mill/week)	Males required for protection of free zones (mill/week)	Total males required whit protection and treatment (mill/week)
1	Years 1-2	755.000	62		62
2	Years 3-4	600.000	45	57	119
3	Years 5-6	500.000	38	45	147
4	Years 7-8	400.000	30	38	178
5	Years 9-10	245.000	20	30	143

Regarding Sicily, cost-benefit analysis showed this hypothesis to be most feasible; so the preliminary project has provided for this productive level in Sicily. However, the estimated productive level will result successful for control programmes, too.

These considerations can only be partially applied to the other Mediterranean Countries; in fact, if the provided strategy will be the same, the methodologies, which lead to the dimensioning, will have to take into consideration the geo-ecological situation of future areas to be treated.

The logical both to be followed for a possible enlargement of the program to Mediterranean Countries, will have to consider the following 9 recommendations as suggested by Kingsley Fisher of the IAEA:

1. as soon as possible make, a decision as to the objectives of a medfly SIT programme: control, eradication or sterile fly sales to the other countries (market assessment);
2. formulate a strategy to adapt the medfly SIT, in a feasible and practical way, to the resources available and to identify the number of years and sources of funding for such a project;
3. initiate a benefit-cost assessment to help rationalise the scope and strategy of the project;
4. initiate a survey programme for the medfly to identify the distribution and density of the pest insect, both geographically and annually;
5. make a choice of which genetic sexing strain to use and initiate a programme to assess its field compatibility;
6. develop an organisation that will be responsible for the conduct of an SIT programme to the total Mediterranean countries;
7. design a facility based upon programme needs, initiating by the productive modules of the planned Sicilian biofactory which allows the production for different medfly genetic sexing strains;
8. initiate a programme of staff training as soon as possible, to include monitoring, mass rearing, quarantine and public relations;
9. a programme of public relations should be initiated.

If we apply the Sicilian data to whole of the Mediterranean area, it's possible forecast a hypothetical productive level of 850 million per week of sterile males which can satisfy a potential request equal to 95% of the whole related to the productive Mediterranean Countries.

Yet Sicily has a natural geo-ecological delimitation, being an Isle. So, in an extremely prudential hypothesis, the above said productive level can be considered equal to 70% of the potential Mediterranean request.

6. THE STERILE INSECT TECHNIQUE AND THE FACILITY DESCRIPTION FOR THE MEDFLY.

The application of S.I.T. medfly has brought the rising of several studies of genetics and molecular biology, in order to sort the sexes too early.
As to the technique used in California, initially, medfly control or eradication programmes were based on the release of both sexes after irradiation. However, the release of sterile females is detrimental to the programme. Although they cannot lay eggs, they attempt to oviposit in ripening fruit. These punctures, called “sterile stings” result in blemishes to the fruit which reduces the price being received by the producer. In addition, mating between sterile males and sterile females reduces the number of matings of the sterile males with wild females.
Independently from the insect species, the technique used, for example, in California reduce the effects of the SIT and, in addition, the economic costs would be too high in terms of necessary materials, facility spaces and work, to release a double amount of sterile insects compared to the requested quantity.
To solve these problems, at the end of 70^{S}, a project followed by FAO-IAEA produced the first Ceratitis capitata strain (WP that is White Pupae) by a mutation of the chromosome X, to allow males to be separated from females at the pupal stage based upon pupal colour.
In fact, at the pupal stage the females are white while the males are brown. In this way, maintaining the same number of larvae in the rearing, the male pupae are sent to the irradiation after the elimination of the females and only adult males are sterilised and then released in the affected areas.
At the beginning of 90^{S}, the laboratory IAEA of Vienna selected a second genetic strain, which showed a double possibility of separation, based on the pupal colour and on the Temperature Sensitivity Lethal (TSL). Exactly by treating the eggs or newly emerged larvae with a slightly elevated temperature (34°C), all females were killed.
The application of TSL to embryonal level, maintaining the same facility plan, results in either an economic reduction equal to 50% of the larval diet and of the spaces, or in an increment equal to 100% of sterile male production.
The technique of the separation of sexes through TSL has only recently solved some problems of stability.
The assumed choice in the planned biofactory, uses the technique of separation of sexes through the pupal colour (WP strain). A system of easy movable walls in the basilar production rooms will permit in a subsequent stage, the conversion of the technique of the separation of the flies through a slightly elevated temperature (TSL strain).
The developed activity of the biofactory consists in the artificial reproduction of the medfly vital cycle. So, all the environmental conditions, conformed to each of the stages related to insect ontogenetic cycle, will have to be reproduced in the facility.

Ontogenetic cycle of Medfy
Photo by FAO/IAEA Agriculture and Biotecnology Laboratory

The planned biofactory will be composed of the following rooms:

1. **Strain back up and filter room**. The rearing of the fruit-medfly in cages subjects them to stress, which causes a gradual phenomenon of genetic sex colour change. In the filter room a pure genetic strain is kept through the adaptation to the gradual stress conditions.
2. **Diet prep.** It's a room connected to the outside and in which the diet for the rearing is prepared.
3. **Colony.** For a production of 85 million sterile males per week, 200 thousand fertile pupae per cage, with sex-ratio 1:1, oviposit the eggs in 130 cages. The collected eggs, mixed with water, are divided into bottles of polyethylene.
4. **Bubbling.** The process lasts 24 hours. The eggs are hatched in the bottles with bubbling.
5. **Seeding room**. The eggs open from the 30th hour to the 60th hour. The eggs are placed in trays containing the diet.
6. **Initiation larvae.** In this room the eggs open and start the maturation process in trays kept in tray-racks.
7. **Maturation larvae.** The larvae stay in this room for 5 days. The larval maturation ends in popping. This is the natural behaviour of the larvae which jump from the fruits to became pupae inside the ground.
8. **Dark room (Pup I).** The larvae are mixed with vermiculite in trays, inside a dark room or a red light room. After a day the pupae are hardened and are divided from the vermiculite through the Vortex, a ventilating separator.
9. **Pupae room (Pup II).** The pupae, separated from the vermiculite, stay in this room for 9 days in trays kept in tray-racks.
10. **Sex sorting room**. After the definitive maturation, the pupae are transferred in this room and separated by colour (white female pupae, brown male pupae) by a machine (the Sortex) normally used for the selection of rice.
11. **Colouring.** In this room male pupae are coloured with an atoxic powder to estimate, after the release in the field, the dispersion of the pupae with a system of traps in the territory.

12. **Irradiation.** The sterilisation of males pupae is carried out by irradiation (ray γ) equal to 150 Gy. The irradiation compromises the functionality of the gonads without alterating the capacity of mating.
13. **Stockage room.** It's a chilled room for the packaging in containers of 4 litres.
14. **Washing room.** The necessary equipment are carefully washed and the diet waste disposal is provided.
15. **Quality control laboratory (QC).** Careful controls are frequently carried out on the eggs production, on the pupae, on the sex, on the capacity of the flight, on the indicator of longevity (long-live), on the indicator of mating.
16. **Staff-room.** It's a place where the staff can relax.
17. **Reception room**. It's the entry or exit place of the biofactory.

The planned biofactory is composed of different and basilar modules, with a productive capacity of about 75 million of sterile males insects per week, including all the abovementioned rooms, except for rooms numbered 11, 12, 13, 14, 15, 16, 17 which are used for several modules.

7. INVESTIMENTAL COSTS

With regard to the costs related to the Sicilian preliminary project, the table below shows the estimated expenses for the realisation of a biofactory with a potential production level of 850 million of sterile insects per week, which will approximately satisfy 85% of the demand required by Mediterranean Countries interested in this technique. The estimated cost amounts to approximately $ US 45,5 million.

	ECONOMIC SUMMARY OF THE PROJECT	
	WORKS	**$ U.S.A.**
	Production area of medfly	
	Production-building	$ 16.577.000
	Outside connections	$ 235.000
	Store	$ 1.325.000
	Office-building	$ 178.000
	Outside placing	$ 3.151.000
A	**TOTAL WORKS**	**$ 21.466.000**
	AVAILABLE EXPENSES OF THE ADMINISTRATION	
	Supplies	
	Irradiation	$ 2.588.000
	Air-conditioning	$ 1.471.000
	Illumination	$ 882.000
	Electric cabin	$ 235.000
	Electric generating equipment	$ 794.000
	Equipments and internal furniture	$ 5.588.000
	Closed circuit television and intercom	$ 71.000
	Alarm system	$ 88.000
	Depurator	$ 706.000
B	**TOTAL AVAILABLE EXPENSES OF THE ADMINISTRATION**	**$ 12.423.000**
	Total (A+B)	**$ 33.889.000**
	V.A.T. (20%)	**$ 6.777.800**
	Acquisition of the area	**$ 371.000**
	General expenses (8%)	**$ 2.711.000**
	Unexpected expenses (5%)	**$ 1.695.000**
C	**TOTAL PROJECT**	**$ 45.443.800**

8. MANAGEMENT COSTS

A biofactory for the production of sterile males of the medfly will have annual management costs equal to $US 26,5 million, including amortisation, routine and extraordinary maintenance, staff, materials, energy, commercial costs, etc..

The break even point of the expenses, each packet consisting of 4 litres of sterile medflies (commercial standard) should be sold for $US 153 equivalent to a production cost of about $US 0,0006 for each sterile fly of the WP strain.

With the above-mentioned productive level, it would be possible to treat about 7,5 million topographic hectares to control/eradicate the medfly over a 211 thousand hectares at risk, the cost amounts respectively to $US 3,6 for a topographic territorial hectare and $US 147 for an hectare at risk.

9. FORMS OF MANAGEMENT

As the insect (fertile or sterile) is endowed with an elevated mobility (helped by winds and by streams it has a range of 100 Km), the farmers are unlikely to buy 4 litres packets of sterile medfly, as the only means for distributing the biological system of eradication is by use of aircraft.
Therefore such a project should be managed by international Organisation, composed by Public Institutions of the Countries interested in the infestation of the medfly.
The participation of the above-mentioned Organisation is evidently a political choice regarding each member, yet, it's important to say that this decision is related to technical aspects (territory, level infestation, necessary years for the eradication, etc..).

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