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ORGANISATION INTERNATIONAL
DE LUTTE BIOLOGIQUE
SECTION REGIONALE
OUEST PALEARTIQUE

INSTITUTO NACIONAL
DE
INVESTIGACIONES
AGRARIAS

REUNION CONJOINTE DES GRUPES DE TRAVAIL

- Lutte biologique contre les revageurs de l'Olive
- Lutte genetique contre *Rhagoletis cerasi*
- Lutte genetique contre *Ceratitis capitata*
- Méthodes génétiques de lutte contre les ravageurs

SASSARI
(Sardaigne, Italie)
15-20 mai 1978

International Organization for
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animals and plants
West Palearctic Regional Section
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La Section Régionale Ouest Palearctique (S.R.O.P.) de l'Organisation Internationale de Lutte biologique contre les Animaux et les Plantes nuisibles (O.I.L.B.) a tenu une réunion conjointe de ses Groupes de travail consacrés à *Ceratitis capitata* Wied, *Rhagoletis cerasi* L., ravageurs de l'Olive et aux méthodes génétiques de lutte, à Sassari (Sardaigne, Italie) du 15 au 20 mai 1978, dans les locaux de la Faculté des Sciences agricoles et de l'Institut d'Entomologie agricole.

Le Conseil de l'O.I.L.B./S.R.O.P. remercie sincèrement Monsieur le Professeur R. PROTA et ses collaborateurs, responsables de l'organisation de cette réunion et d'une excursion dans la région de Bad de Salighes et de Villasor et les responsables des différents Groupes de travail E. BOLLER, U. CIRIO, L. MELLADO et R.J. WOODS qui ont assuré l'animation scientifique des sessions.

Cette réunion conjointe a permis de dégager la nouvelle politique scientifique de l'O.I.L.B./S.R.O.P. dans le domaine de la lutte biologique contre les mouches des fruits, suivant les recommandations proposées par les participants au Conseil de l'O.I.L.B./S.R.O.P. qui les a adoptées lors de sa réunion annuelle des 9 et 10 novembre 1978.

P. FERRON
Secrétaire Général de l'OILB/SROP

b) Genetic methods

c) *Rhagoletis cerasi*

INTRODUCTION

The works of the meeting have been carried out in four plenary sessions (where have been presented and discussed the general reports) and in four separated sessions (where have been presented and discussed the particular reports) and where, on the base of the attained results in different fields of study, the theoretical and practical programmes of future researches were co-ordinated.

In chronological succession have been treated the following general topics :

1. Genetical and biological control against the fruit flies and the noxious insects of olive ;

2. Biotechnical methods of control with particular care to the attractants and to the repellents and to the genetical processing ;

3. The quality control of noxious insect to the fruit-trees and of useful insects.

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ADDRESS BY THE HON. MR. ERDAS

Ladies and Gentlemen, it seems superfluous to me, in my role of Environmental Protection Assessor for the Region of Sardinia, that I should emphasize the great importance and value or the scientific, ecological and cultural significance of this meeting, which brings together, here today in Sassari, experts, researchers, professors, and Regional Administrators. So, it is in full awareness and certainly not out of formality that, in the name of the Regional Administration, the Regional Council, the President, M. SODDU, and in my own name, I wish to express appreciation, the greatest appreciation, for this initiative that, as I was saying, has caused to be assembled the major experts in the international field for the purpose of comparing and confronting, during four strenuous days of study and debate, the very urgent problems of insect pest control that exist in agriculture today.

I express appreciation, therefore, and thanks to the Rector, to the Director of the Faculty of Agriculture and, in particular, to Prof. Romolo PROTA, Director of the Institute of Agricultural Entomology, not only for wanting and succeeding in organising this extremely important meeting in Sardinia, but for his work and results obtained, and for his energy and, I would add, constancy in pursuing helpful scientific co-operation with other research and experimental bodies and institutions ; but also thanks for his unremitting efforts to arouse and interest the public administration, so often deaf, indifferent or unfeeling towards such important research and experimentation in this essential and most delicate field of controlling insect pests while, above all, preserving our natural environment. Today, we wish to acknowledge such dedication and effort, and such continued attempts to obtain collaboration that, inexplicably, other institutes, even at university level, do not always demonstrate in deeds ; they neither seek it nor practise it. And, in this respect, I wish to mention the most helpful co-operation with the agricultural section of CRAI, run by our indefatigable Carlo CONTINI, and the research and other work carried out together ; and also with the Phytopathologic Observatory, under our eminent Prof. ZANARDI, for years carrying on experiments, research work and trials of pest control methods that avoid pollution of the natural environment with insecticides, or reduce it to acceptable limits. Often, and not off the point, since it is by now part of the pollution "story", one hears of synthetic organic substances being employed in agriculture which, besides not giving the hoped-for results, have determined the selection (as you certainly know) of resistant strains ; and, as a consequence, increasingly greater quantities of more and more toxic products have been employed, causing large areas to be heavily contaminated, often irreparably, due to the complete unbalance and deterioration of the entire eco-system.

There is now urgent need for a fully developed awareness that pest control and plant protection in general have to be directed towards the various methods of biological control, so that every form of environmental pollution will be at first contained and finally completely eliminated. It is, indeed, because our aims, ideas and lines of action coincide -because of our common interests- that we warmly welcomed the proposal of the Institute of Agricultural Entomology to hold such a highly authoritative congress in our island, where agriculture is directly involved in solving the problems concerning the control of insects particularly harmful to the basic crops produced in the whole of the Mediterranean.

We are grateful, therefore, that you are honouring us with your presence and are putting at our disposition the benefits of your experience and studies, and the results of your researches and experiments. We are convinced, moreover, that this meeting offers with the experience and specific competence of the highly qualified experts present, a unique opportunity of formulating biological control programmes to be carried out in this island, which, we think, in view of its actual insularity, is most suitable for operations of this nature. We also believe that aid for such a scheme may be obtained from the Development Fund of the European Community and from those national and international bodies operating in these fields.

The Regional Administration categorically confirms today its complete readiness and commitment to lend its own organizatory and operative machinery in order to promote and facilitate the application of these new techniques, which are, of course, the subject of these highly important discussions. The simple wishes and hopes that we now express are that this congress, your labours, will have every success in finalizing plans that will indeed secure our agricultural production, but will, above all, secure the environmental patrimony which is ours, and which we all desire and intend to preserve.

ADDRESS BY PROF. R. PROTA

Mr. Secretary, Ladies and Gentleman, before begining my lecture allow me to express my sincere pleasure in welcoming you to this meeting, and my gratitude to everyone who has helped in bringing it about.

I remember that here, exactly three years ago (with a Working Group on *Ceratitis capitata* Wied.) we went into the question of applying genetic control techniques in Sardinia.

Today, we have to deal with other kindred problems. But, I am sure that, from the exchange of ideas and information among the various sectors gathered here this week and even arising from the very problems existing in our island there will ensue solutions acceptable by all interested parties.

I know that the representatives of our Regional Administration present in this hall will realize the urgency of the problems and solutions proposed, and will readly appreciate haw they may apply to our Island both from an economic and social point of view, as well as from that environmental conservation.

INTRODUCTION TO THE FRUIT FLY SITUATION IN SARDINIA

Romolo PROTA

(Istituto di Entomologia Agraria - Unisersita di Sassari)

The development of modern fruit cultivation in Sardinia is subject to the orography and pedoclimatic conditions particular to the island. Frequent north-westerly winds and hot tropical air masses from the south have a strong influence on the climate, which frequently varies from one part to another.

Rainfall is low (an average of 700 mm), the greater part of which occurs in autumn and winter, usually following a long period of dryness during summer.

The temperature is typical of the mediterranean basin but, in addition, it suffers sudden fluctuations both in winter and spring.

Under such climatic conditions efficient production can only be obtained with the aid of irrigation.

Present fruit cultivation covers only a tenth of the total surface area (about 2 million hectares), a fifth of which consists of flat country, and a half of highlands.

The foregoing brief points are only of general nature, but it can be affirmed that there is still much more room for further development of this type of cultivation, especially when one considers, on the one hand, modern technical innovations, and on the other the greater consumer demand due to population increase and rise in the standard of living.

But, if we are to realize such an important development without suffering premature or subsequent disappointments, it is essential that all means available for this purpose are programmed in accordance with certain indispensable technical criteria including plant protection.

Determination of the most suitable growing regions and methods for each main type of cultivation cannot be divorced from social and economic considerations. The effects -and I repeat- both social and economic must be accurately evaluated before deciding which metnod to adopt of controlling parasitic infestation.

With regard to the fruit fly, our fruit cultivation operates under certain difficulties due, on the one hand, to ancient traditions such as the sub-division of land tenure and, on the other, to more recent causes such as irrational planting, inadequate pest-control techniques, and so on.

Rhagoletis cerasi Linné

Cherry cultivation is found over practically the whole of this island, but particularly, concentraded in 4 localities (Bonnannaro (SS), Belvi (NU), Villacidro (CA) and Burcei (CA)). The total area covers about 600 ha (almost all being mixed cultivation) producing approximately 11.000 quintals a year, which, up to a few years ago, was equal to the internal demand.

Attacks by *R. cerasi* L. are most severe in the central regions (Villacidro and Belvi). In the higher altitudes (Gadoni for instance), the insect adult (observed by yellow coated traps) is generally present for about two months, that is from mid-May to mid-July, damage being first noticed when early cherries are picked at the end of May.

In the Villacidro region, control is effected with Dimethoate, and production losses may be from 10 to 20 %. In the region around Belvi, control measures are not usually carried out, and production losses may be from 30 tà 40 %. In the whole of the island, about 6 % or production is lost, amounting to 700 quintals, or 70.000.000 lires.

Dacus oleae Gmelin

Olive growing has been a traditional occupation in Sardinia since the earliest times and is the second largest arboricultural activity, both in area covered (more than 50.000 hectares) and overall productivity.

The groves are not distributed over the island homogeneously, but are found mainly in the North. These larger concentrations are responsible for about 50 % of the total island production, and give rise to a whole series of economically important ancillary activities. Management cannot be called highly rational. There are other adverse factors, such as the unsuitability of much of the land occupied, irregular and defective planting, dissimilarities between one tree and another, differences in ripening periods - all of which contribute in reducing the average return to the producer. If we then add inefficiency in harvesting, storing and processing (resulting ofter in poor quality oil of high acidity) together with an ever increasing cost of labour, it can be seen that the industry is in jeopardy.

Reduction of production costs and improvement in oil quality are the principal remedies for survival.

At this point, we earnestly appeal to all interested parties to examine carefully the present situation and, as previously mentioned, adopt more and more rational and efficient methods of management, always bearing in mind the fundamental principles of environmental conservation.

Plant protection, I repeat, must be based on new techniques. For more that 20 years, anti-Dacus measures in Sardinia have been carried out using the same chemical agent in continually increasing doses.

The consequences are readily intuitable-breakdown of biological relationship between plant and biocoenosis together with the probability of having caused resistent strains of the pest.

This situation will certainly become worse when the groves are extended towards more fertile and more easily irrigated land and the fruit will be subject to increasingly more severe infestations.

In this respect, it seems necessary to deal with certain technical scientific aspects regarding the most predominant olive phytophage before being able to formulate a control programme, which could also be used against other pests.

Particular emphasis has been laid on those key factors considered indispensable in scientifically evaluating forecasts of the attacks, that is, firstly the dynamics of the insect population, and secondly, the mechanisms of population regulation.

We know that the imago of *Dacus oleae* Gmelin is present in Sardinia the whole year round, with peak periods at the time of the spring and autumn generations. The pupa hibernates in the ground, and all larval instars in the hanging drupes.

Table cultivars (growing in the deep-soiled, coastal plains) are the first to be attacked ; then follow the oil cultivars (growing in the shallow-soiled, inland highlands). These attacks are made by an uninterrupted series of 4-5 generations in autumn and 1-2 in spring.

Population totals vary during the course of the year due to various factors, which have different effects from zone to zone.

In the large northern groves (that is Alghero, Sassari, Sorso, Ossi and Bosa) the population dynamics and development of the infestations seem to be greatly affected by the simultaneous action of three basic factors.

1) The size of the crop and lenght of time it remains on the tree.

2) The amount and extent of rainfall, especially in spring and summer.

3) Temperature extremes during summer and winter.

Then, there are many other factors which also contribute to determining the intensity of the attacks during the year.

For example,

a) the weight of the initial attack ;

b) average temperature and humidity ;

c) period when the fruit is most vulnerable ;

d) the predatory factor during pre- imaginal stage in the ground.

Without going into details of the intricate complex of factors which regulates the populations, I cite two examples in which the size of the crop directly influences the numbers of the insect together with the other factors already mentioned.

These two cases, are not exceptional, but, as the oil cultivar crop in our island alternates from year to year, they produce opposite and often unexpected results.

CASE 1) In a year of poor yield (under 7 kg per tree) - In these circumstances, the attack by *Dacus* may cause an almost total loss of fruit, the consequent annulment of the spring generations, and thus only minor damage to the abundant subsequent crop. But, only, if temperatures exceed certain limits (to be precise, over 30°C in summer, under 20°C in autumn and under 0°C in winter) and if rainfall during spring and summer is low. However, in simular conditions of temperature, but where the June and July rainfall exceed an average of 50 mm, very high levels of infestation occur - due to early ripening and vulnerability of the drupes.

CASE 2) In peak years (that is when the average yield per plant exceeds 40 kg). Here infestation is relatively insignificant, provided that climatic conditions follow their normal course. Naturally if picking is delayed until spring, the *Dacus* populations increase.

Regarding biotic factors, *Dacus* suffers considerable predatory damage while hibernating in the pupal state. This is especially so in a mild winter or in low production years. Restriction of the populations by parasitic entomophages in somewhat limited (Tab. 1).

Ectophagic calcidids are known to be present, namely : *Pnigalio mediterraneus* Ferr. et Del., *Eupelmus urozonus* Dalm., *Euritoma martellii* Dom., as well as the dipter *Prolasioptera berlesiana* Paoli.

Tab. 1 - Parasites of Dacus oleae observed in Sardinia during the years 1973-77

Species	Years				
	1973	1974	1975	1976	1977
Pnigalio mediterraneus Ferr.	+++++	+++++	+++++	+++++	+++++
Eupelmus urozonus Dalm.	+++	+++	+++	+++	+++
Euritoma martellii Dom.	++	+	++	+	+
Prolasioptera berlesiana Paoli	+	+	+	+	+
Opius concolor Szepl			+		+

Opius concolor Szepl is found mainly in the south. In some parts (around Teulada, for example) where the *Dacus* populations are fairly thickly concentrated, the parasitic incidence of *Opius* reaches fairly high levels -more than 35 %. The most northerly region in which *Opius* has been observed is around Bosa, where an attempt was made to colonize the area in 1974. Examination of drupes (40 % infested) in early autumn showed that this braconid parasite occurred in 20 % of the *Dacus* populations.

Tab. 2 - Olive production losses through Dacus oleae infestation (average of two years)

Cultivation type	Years	Effective yield (Quintals)	Presumed loss			
			%	Quintals	It. Lire x 1,000,000	$
Specialized and Mixed	1953-54 1954-55	530,000	37.60	319,170	12,128	14,269,000
Specialized and Mixed	1974-75 1975-76	581,700	18.80	109,360	4,155	4,890,000

It is difficult to arrive at a scientific evaluation of the damage suffered by the olive growing industry due to *Dacus* alone, but we have made an approximate calculation for the period 1974-76.

In table 2, the data for this period are compared with those obtained from similar researches carried out from 1953 to 1955 and are sufficiently reliable to give a clear picture of the difference between the two periods. To the losses of production are also to be added those due to the inferior quality of the oil. Bearing in mind the fact that two or even three application of anti-dacus treatment are employed every year, and that the dosage is continually increasing, the financial losses suffered by the olive-growers are becoming undoubtedly difficult to bear.

Ceratitis capitata Wied.

The fruit-growing industry in Sardinia is traditionally based on the small family type of orchard. However, rational cultivation has been increasing over the last twenty years. The orchards are usually mixed both as to type and variety - such fruits as apples, pears, peaches and apricots being planted indiscriminately together with plums, cherries, figs and persimmon.

The problem of deling with *Ceratitis capitata* Wied. is complicated by this type of fruit cultivation. Possible solutions depend as much on the way orchards are generally planned as on following correct control procedures regarding each individual species concerned.

Thus, although the industry here has the advantage of an early production and is, indeed, still expanding, there are, on the other hand, the disadvantages arising from present methods of planting, or rather lack of method.

Even if the situation is gradually improving, after only a brief study of a map showing the principal growing areas (subdivided into single type and mixed type cultivation) we soon realise the manifold difficulties still to be encountered in effecting a modern plant-protection programme, difficulties not lessened by the presence of wild host-plants so widespread as to be practically uncontrollable.

The main centres of fruit cultivation, as can be seen on the attached map, are found in the central and southern regions of the island, where citrus and peach production predominates. Citrus plantations amount to a total area of about 10,000 hectares and peach plantations to about 3,000 hectares. Other fruits are relatively of minor importance, although the apricot and pear, both as to number and diffusion of plants, deserve mention.

It should be emphasized that a systematic accumulation of data regarding the effects of *Ceratitis capitata* infestation

has only recently been completed, through the help of the Regional Anti-insect Centre (Centro Regionale Antinsetti-GRAI). This body enlarged on the work started by the Research Section of the National Committee for Nuclear Energy (CNEN).

The main objectives were to define :

1) the area in which the insect may create a real hazard ;
2) the frequency with which control measures need to be applied ;
3) the production losses caused by the attacks.

Method followed were similar to those adopted in the case of *Dacus*, and population dynamics monotoring was effected by means of attractant boards.

In the milder regions, *Ceratitis capitata* Wied. is found in the larval stage throughout the year. The incidence of infestation month by month is shown in table 3.

Tab.3 - Larval stage of C.capitata in various hosts present in Sardinia

Host	JAN.	FEB.	MAR.	APR.	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.	DEC.
Bitter Orange	———	———	———	———								
Sweet Orange		———	———	———	———							
Valencia late				———	———	———	———					
Clementine									———	———	———	
Mandarine									———	———	———	
Lemon							———	———	———			
Grape fruit									———	———	———	
Peach					———	———	———	———				
Apricot					———	———	———					
Fig					———	———	———	———	———			
Pear						———	———	———				
Apple									———	———		
Kaki (persimmon)									———	———		
Prickly pear									———	———	———	———

As the investigations proceeded from zone to zone in the course of the 4 year programme, population density and dynamics varied considerably, according to ambient characteristics, pedoclimatic conditions, cultivation methods, and -as mentioned before- according to extent to which host-plants may have been present.

Generally speaking, population densities are observed to be progressively greater from north to south, and while the more northerly regions are only attacked in August and September, the more southerly suffer all the year round. This general situation, however, can be modified by local climatic conditions which differ from the norm, as, for example, in certain north-central regions, where typically southern fruits have been introduced.

Regarding the population dynamics of this insect in Sardinia, fluctuations are considerable, but studies have yet to be completed before the numerous factors involved can be satisfactorily explained.

Ceratitis capitata, then, is a constant menace to all fruit trees in the more southerly regions. It attacks the mixed groves of Calasetta, S. Antioco, and Domus de Maria-Chia ; the peach groves of S. Sperate and the citrus groves of Muravera, Milis, S. Vero Milis, where there are the more vulnerable species and cultivars (or later ripening varieties, such as the orange *Valencia late*).

The year 1977-78, although not yet concluded as regards certain citrus species, is already showing the effects of an outbreak more severe than in the three previous years. This increase in the *Ceratitis* population is basically due to unusual climatic conditions. Above average spring and summer rains provided the insects with earlier and more abundant nutriment ; and the extended mild season (up the end of November) facilitated and prolonged their activities. The intensity of the attack (expressed by the weekly average number of adults attracted during the major vulnerability period of the fruit) and yield losses, calculated from fallen or otherwise unsaleable fuit, are shown for citrus fruit and peaches in two diagrams.

Details regarding citrus fruit, normally treated with two applications of Dimethoate, are as follows :

In the province of Sassari (in groves around the city) the average number of insects attracted was 22 per attractant board. Yield losses were 0,5 % and quality depreciation 7 %.

In the province of Nuoro (in the area of Siniscola), insects attracted averaged 345, yield lost came to 1,9 % and depreciation 30 %.

In the province of Oristano (at Milis-) insects attracted averaged 46 ; yield losses were 2,6 % and depreciation came to 8,5 %.

In the province of Cagliari (at Monastir, Decimomannu and Muravera) an average of 26 adults was registered ; yield losses were 1,9 % and quality depreciation was 16 %.

With regard to the peach, Northern Sardinia (Sassari) showed a weekly average of 1 adult per attractant board and a yield loss of 20 %, whereas in the South, adult insects attracted came to 7, with yield losses of 25 %.

It would seem possible to state that financial losses in Sardinia resulting from fruit-fly attacks of medium intensity such as in 1977-78 amount to about 4,000,000,000 lire ; including just over 1,000,000,000 lire for citrus fruits and roughly 2,500,000,000 lire for peaches (Tab. 4).

Tab.4 - Approximate fruit production losses in Sardinia due to Ceratitis capitata attacks (1977).

Type of fruit	Effective production (quintals)	Estimated yield loss		Quality depreciation		Estimated financial loss	
		(%)	(quintals)	(%)	(quintals)	in Lire x 1.000	$
Orange	442,034	0.7	3,168	21.5	95,034	750,156	872,274
Clementine	9,923	46.7	8,701	-	-	348,078	404,741
Mandarine	136,000	0.5	750	-	-	14,990	17,430
Peach	119,045	23.9	37,557	-	-	2,628,990	3,056,965
Apricot	11,661	5.0	648	-	-	32,400	37,674
						3,774,614	4,389,084

The situation may, in fact, be more serious ; for, we have not taken into account the losses suffered by that part of the industry which grows fruit of secondary importance. In addition, there have to be considered the increasing expenses due to the more and more frequent use of insecticides, not always giving effective results.

In this respect, it must be mentioned that, from data collected on the consumption of insecticides in Sardinia, facts of extreme gravity have emerged regarding both the excessive economic cost and the deleterious effects on the environment. The amount spent on protecting just the olive, peach and citrus fruits against Tripetid attack is in the region of 1,000,000,000 lire (Table 5).

Tab.5 - Approximate costs of protecting olive and main fruits in Sardinia against Dacus oleae and Ceratitis capitata.

Product	Insecticide		Labour cost	Total cost	
	Quintals	Lire	Lire	Lire	$
Olive	556	166,805000	310,443,000	477,249,000	554,940
Citrus	491	147,315000	343,735,000	491,050,000	570,988
Peach and Apricots	180	24,185000	56,431,000	80,616,000	93,739
				1,048,915,000	1,219,667

CONCLUSIONS

Generally speaking in the situation does seem to be showing some signs of gradual improvement in techniques. Dut it is difficult to understand why certain growing projects were undertaken without sufficiently considering the plant-protection aspects or the question. The total losses suffered in the sectors already mentioned amount to nine thousand million lire about 26 % of the value produced.

An immediate solution would appears to be extremely doubtful. For, on the one hand, more and more numerous chemical products are being increasingly used (without proper guidance, trough insufficient numbers of advisory control bodies) ; and, on the other hand, there is still not enough awareness that control methods are required more adequate to the situation.

I, therefore, take the opportunity given in this session, of urging everyone engaged in improving our main commercial fruit industries to expedite their work, not forgetting that production costs must be limited as much as possible.

I also urge that more suitable plant-protection methods be adopted, and that they become continually more efficient for every point of view.

The essential of any scheme for modernising plant-protection in our fruit-growing industry can be stated thus :

- the choice of varieties to be planted must depend on the vulnerability of their fruit to attack ;

- before planting fruit-trees or new cultivars, the "typical growing areas" must be studied, and the composition and density of their fauna estimated, cultivation techniques must be revised, so as to minimize the danger of attacks ;

- a growers'plant-protection association must be formed.

More specifically regarding the olive, better results can be achieved by :

1) timely picking and mechanisation ;

2) exploiting the plants natural tendency to alternate annual cropping ;

3) by defining sampling methods and the economically tolerable level of the attacks.

With regard to *C. capitata*, while waiting for the completion of studies which will enable protection measures to be adopted more in keeping with modern concepts of biological control, it would seem necessary to revise all present practice if we are to avoid an absurde waste of money, environmental adulteration, and harm to Man and other animals.

Under the circumstances, I consider it essential to urge closer ties between state research entities and Regional Institutions, with the object of extending experimental knowledge and to resolve two fundamental requirements :

firstly, the need to demonstrate the validity of the new control methods, especially in the field ;

and, secondly, to create qualified technicians to distribute correct and up-to-date information on non-polluting means of protection.

CONTROL GENETICO DE *Ceratitis capitata* WIED, EN LA ISLA DEL HIERRO (ISLAS CANARIAS)

J.P. ROS

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Desde 1965, el Instituto Nacional de Investigaciones Agrarias (INIA) del Ministerio de Agricultura de Espana, en colaboración con el OIEA, viene desarrollando un programa de lucha biológica contra la mosca de la fruta (*Ceratitis capitata* Wied.) por el método de "insectos estériles" (SIT). (1, 2, 4).

Ensayos preliminares en la isla de Tenerife de 1966 a 1968, (3, 5, 6) ; en la provincia de Murcia, 1969 (7, 8, 9, 10, 11) ; y en la provincia de Granada 1971 a 1973 (12, 13, 14, 15, 16, 17) ; han demostrado que el método es plenamente eficaz aplicado a zonas de reducida extension y relativo aislamiento.

Antecedentes

Desde 1974, se viene desarrollando el programa de lucha biológica contra *Ceratitis capitata* en la Isla del Hierro (Islas Canarias) Fig. 1 (18, 19, 20, 21). Este programa incluye el estudio de las variables climáticas, edáficas, ecologicas y poblacionales de *Ceratitis* en esta isla.

En los trabajos realizados en 1974/75, se obtuvieron las siguientes conclusiones :

a) Solamente se registró la presencia de *C. capitata* Wied. en la zona denominada "El Golfo" en la vertiente norte de la isla, en la que abundan los cultivos de melocotón, aguacate, platano guayaba, vid e higuera.

Esta zona está totalmente aislada del resto de la isla en su vertiente norte por el mar y en su vertiente sur por un acantilado de 1.200 metros de altura media.

b) La población adulta de *C. capitata* alcanza su maximo en elmes de octubre, registrándose capturas de 20 moscas/mosquero/dia.

c) El máximo de ataque se registra en el mes de julio-agosto llegando a porcentajes madiös muy altos (30-40 %) de infestación de fruta.

d) El fruto que mas acusa al ataque de *C. capitata* as el melocotón que madure escalonadamente desde abril a agosto, pudiendo acortarse este periodo si las temperaturas en primavera se mantienen por debajo de lo normal.

e) En invierno, la poblacion adulta de *C. capitata* es muy escasa o nula no registrandose capturas desde diciembre hasta abril.

La población larvaria, pasa el invierno en los frutos cítricos y quizá en la chumbera (Opuntia ficus indica) muy abundante en la zona, aunque este ultimo hecho no ha podido establecerse - de forma clara.

Material y métodos

En 1975, la liberación de insectos estériles en la zone de "El Golfo" comenzó en abril, al detectarse las primeras capturas de *C. capitata*, prolongandose hasta septiembre. Se liberó un total de 107 millones de pupas irradiadas a 9 Krad y procedentes de la unidad biologica de cria masiva de *C. capitata* del INIA en El Encin (Madrid), registrandose una eclosion media del 60 %.

En 1976, se liberaron en la misma zona un total de 87 millones de pupas irradiadas, con una eclosion media de 67 %. Por falta de recursos economicos se suspendieron las liberaciones de insectos a partir del mes de septiembre.

El transporte de pupas, en estos dos años, se efectuó en avión, en recipientes de madera con malla metálica para facilitar la aireación a razón de 3 envíos semanales. En ambos años al final de la campaña de liberación de insectos estériles, se efectuó un estudio de la población indígena residual en la zona

En 1977, se cambió el sistema de transporte, sustituyendose las cajas por bolsas de plástico. En los ensayos efectuados en el laboratorio, las pupas de *Ceratitis* introducidas en bolsas de plastico (poliestileno) cerradas al vacio o en atmósfera de nitrógeno, a razón de 50.000 pupas/bolsa, pueden sobrevivir perfectamente hasta 6 dias en este ambiente. La variación de la eclosión va desde el 85 % (igual al testigo) a las 24 horas de tratamiento, al 50 % de eclosión al 6° dia.

Este nuevo sistema ha permitido una reduccion considerable de los costos de transporte asi como una ostensible mejora en la manipulación . La eclosión en la zona experimental es del 80 % de media. Hasta el mes de noviembre se liberaron 90 millones de insectos estériles y se pretende liberar todo el año.

Resultados

En el Cuadro I, se recoge la prospección de la población de *C. capitata* en el año 1974.

En los Cuadros II y III, se recoge las poblaciones indígenas residuales, después de la aplicación del SIT.

En los Cuadros IV, V y VI, se recoge el control de la fruta en los años 1975, 1976 y 1977.

La Figure 1, es el plano de situación de la isla del Hierro (Islas Canarias).

La Figura 2, es la gráfica correspondiente al Cuadro I.

La Figura 3 y 4, son las gráficas de control de la fruta de los años 1975, 1976 y 1977.

La Figura 5, es la representacion de las poblaciones residuales indígenas de los años 1974, 1975 y 1976.

Conclusiones

a) Como se aprecia en los Cuadros I, II y III y en sus correspondientes Figuras (3 y 4), la maduración de la fruta a causa de condiciones climaticas, tuvo un desplazamiento en el tiempo en el ano 1976 respecto a 1975.

Asi en el año 1975, el máximo de cosecha se recolectó en los meses de mayo y junio, quedando muy poca fruta al principio del mes de agosto.

Por otra parte en 1976, el maximo de cosecha se recolecto en los meses de julio y agosto, quedando bastante fruta a finales del mes de septiembre.

En 1975, se dejó de liberar insectos estériles el 17 de septiembre, las últimas generaciones de *C. capitata* tuvieron lugar en el mes de agosto, con lo que se tuvo el plazo de un mes para combatirlas con el método de "insectos estériles".

Por esta razón la prospección de la población indígena residual efectuada en los meses de octubre y noviembre dieron valores muy bajos (Fig. 5).

En 1976, por razones económicas se dejó de liberar insectos estériles el dia 23 de septiembre cuando precisamente quedaba fruta aún en los árboles y en plano auge (temperaturas altas) las sucesivas generaciones de *C. capitata*. La respuesta de la prospección de la población indigena residual efectuada, lo mismo que el año anterior en los meses de octubre y noviembre, fue obvia, los valores crecieron a límites bastantes altos (Fig. 5).

Por este razon en el ano 1977 se liberaron insectos estériles durante todo el ano, hasta enlazar las sucesivas campanas.

La experiencia anterior puede corroborar en algo la eficacia del método de los "insectos estériles".

b) Es de señalar también que en las tres campañas (75, 76 y 77), effectuadas en esta zona experimental nos hemos movido en nivales de esterilidad (moscas estériles/Ha) muy bajos, ya que dicha zona ocupa una extensión aproximada de 3.500 Ha. Nuestros envíos semanales oscila alrededor de los 5 millones de insectos estériles lo que nos da una media de 1.100 moscas estériles/Ha, cuando lo recomendable suele ser de 10.000 moscas estériles/Ha.

Por lo tanto, la erradicación de la plaga no ha sido posible, sin embargo, el control de la misma dá unos resultados excelentes.

Resumen

El Instituto Nacional de Investigaciones Agrarias, viene desarrollando desde 1974 un programa de erradicación de *Ceratitis capitata* por el método de "insectos estériles" en la isla del Hierro.

Aproximadamente 100 millones de pupas de *C. capitata* se liberaron en cada campaña (75, 76 y 77), en la zona denominada "El Golfo" de aproximadamente 3.300 Has.de extension.

El control de la fruta (melocotón) contra el ataque de este insecto had ado en las tres campañas resultados muy aceptables, al mantenerse el ataque medio por debajo del 1 %.

La erradicacion del insecto sin embargo, es más problematica, debido al escaso nº de insectos estériles liberados para la amplia zona a erradicar, estando en un 10 % del nº optimo de insectos estériles/Ha.

Summary

Since 1974 the National Institute of Agronomic Research (INIA) has been carrying out a program for the biological control of *Ceratitis capitata* Wied. by the Sterile-Insect Technique, on the Island of Hierro (Canary Islands).

Approximately one hundred million irradiated pupae were released every year (1975-76-77) in the experimental area of about 3.300 Ha.

The check of the test fruit (peach) infested with *C. capitata* showed that infestation stayed always below 1 %, which can be considered quite satisfactory. Thus, the use of the S.I.T. has been shown to be a good control method. However to achieve erradication of the pest, releases would have to be increased considerably.

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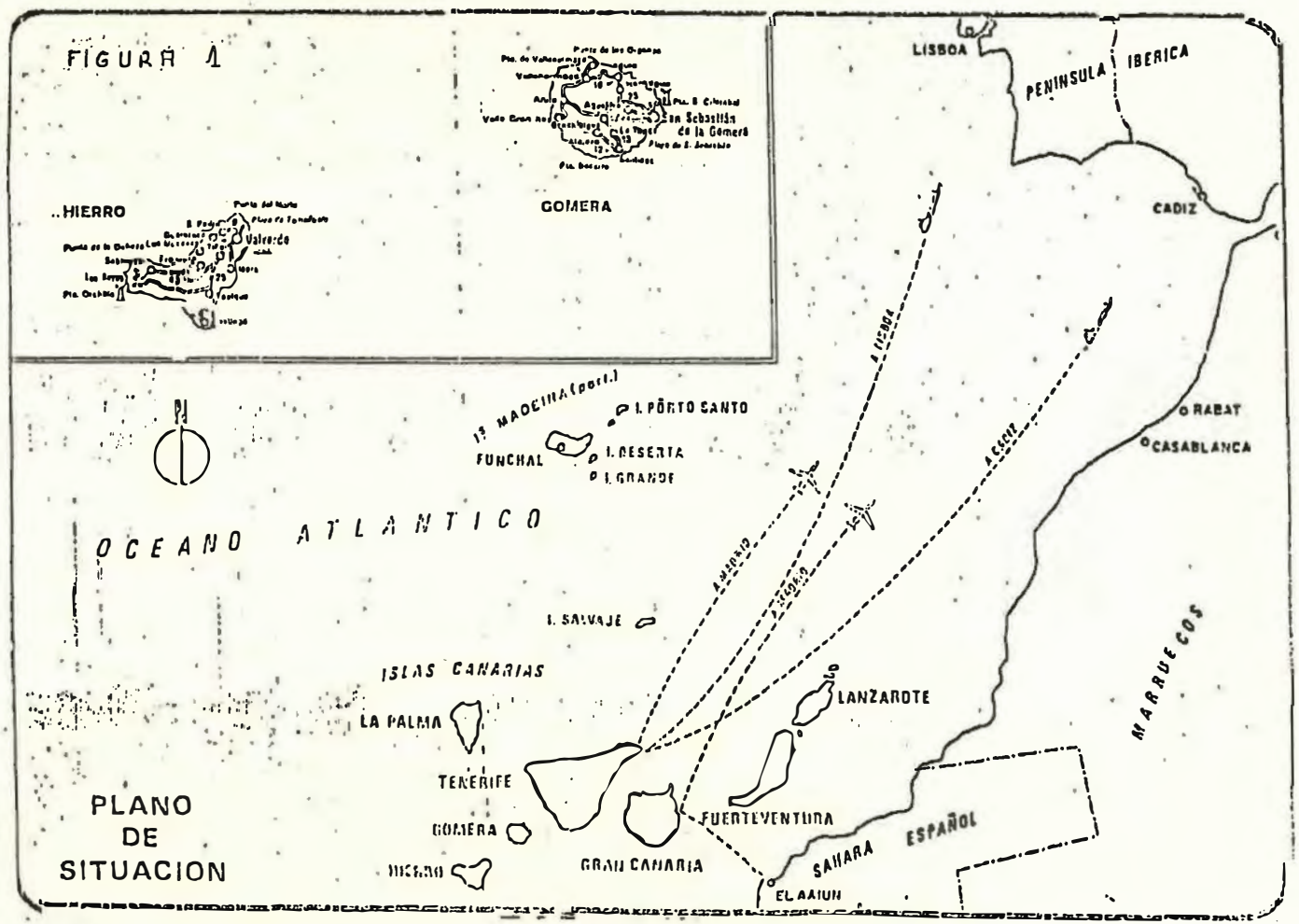
FIGURA 1



PLANO DE SITUACION

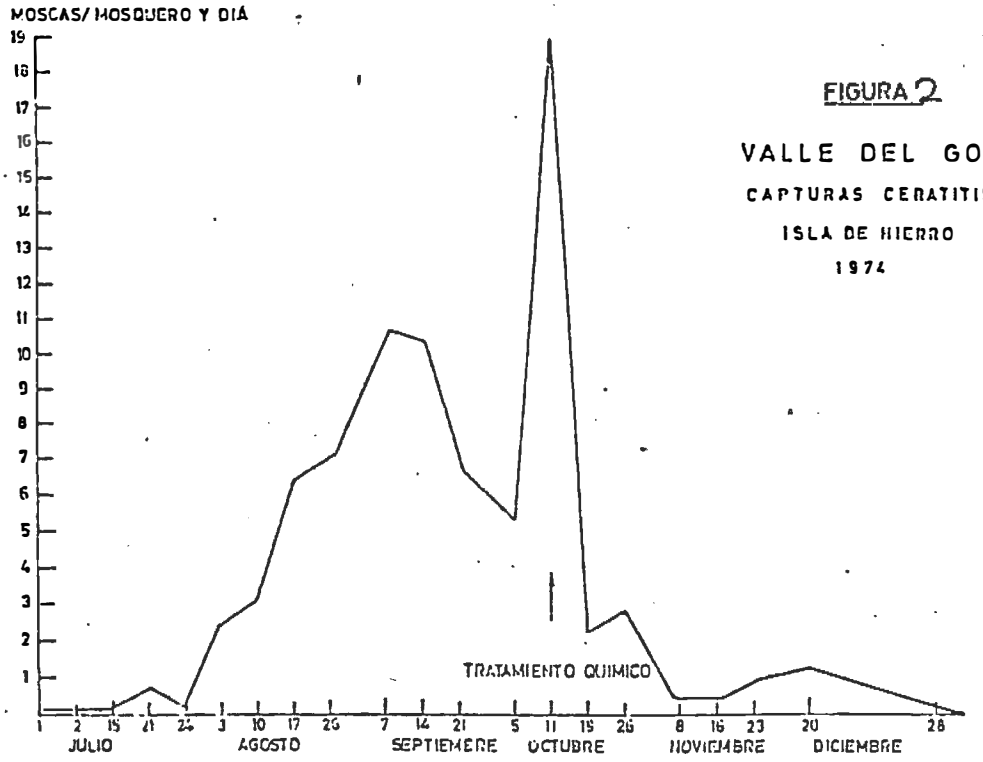


FIGURA 2

VALLE DEL GOLFO

CAPTURAS CERATITIS

ISLA DE HIERRO

1974

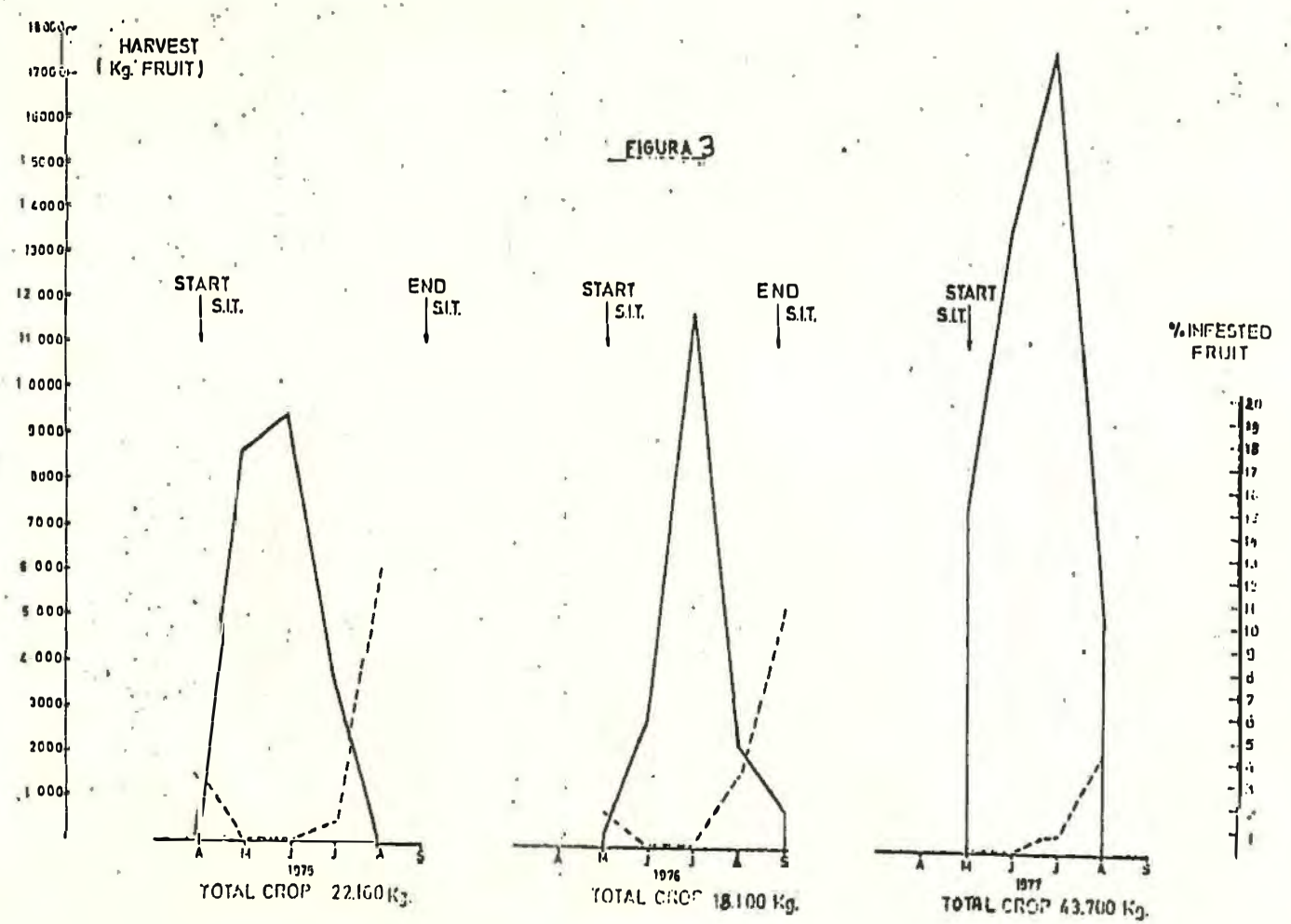
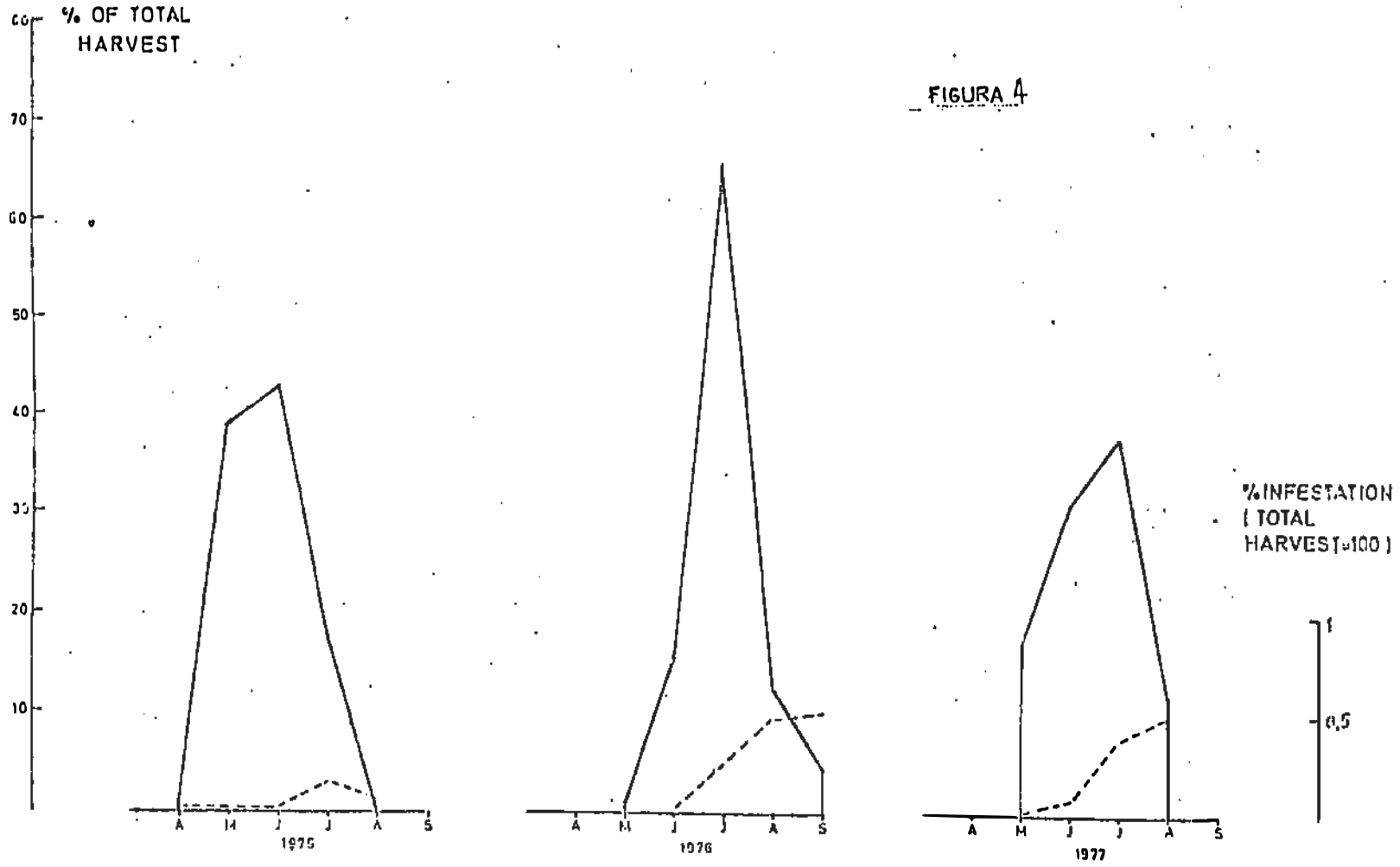

HARVEST
(Kg. FRUIT)
START
S.I.T.
END
S.I.T.
1975
TOTAL CROP 22.160 Kg.
START
S.I.T.
END
S.I.T.
1976
TOTAL CROP 18.100 Kg.
START
S.I.T.
1977
TOTAL CROP 43.700 Kg.
% INFESTED
FRUIT

FIGURA 3

FIGURA 4


% OF TOTAL HARVEST
70
60
50
40
30
20
10
A M J J A S
1975
1976
1977
%INFESTATION (TOTAL HARVEST=100)
1
0,5

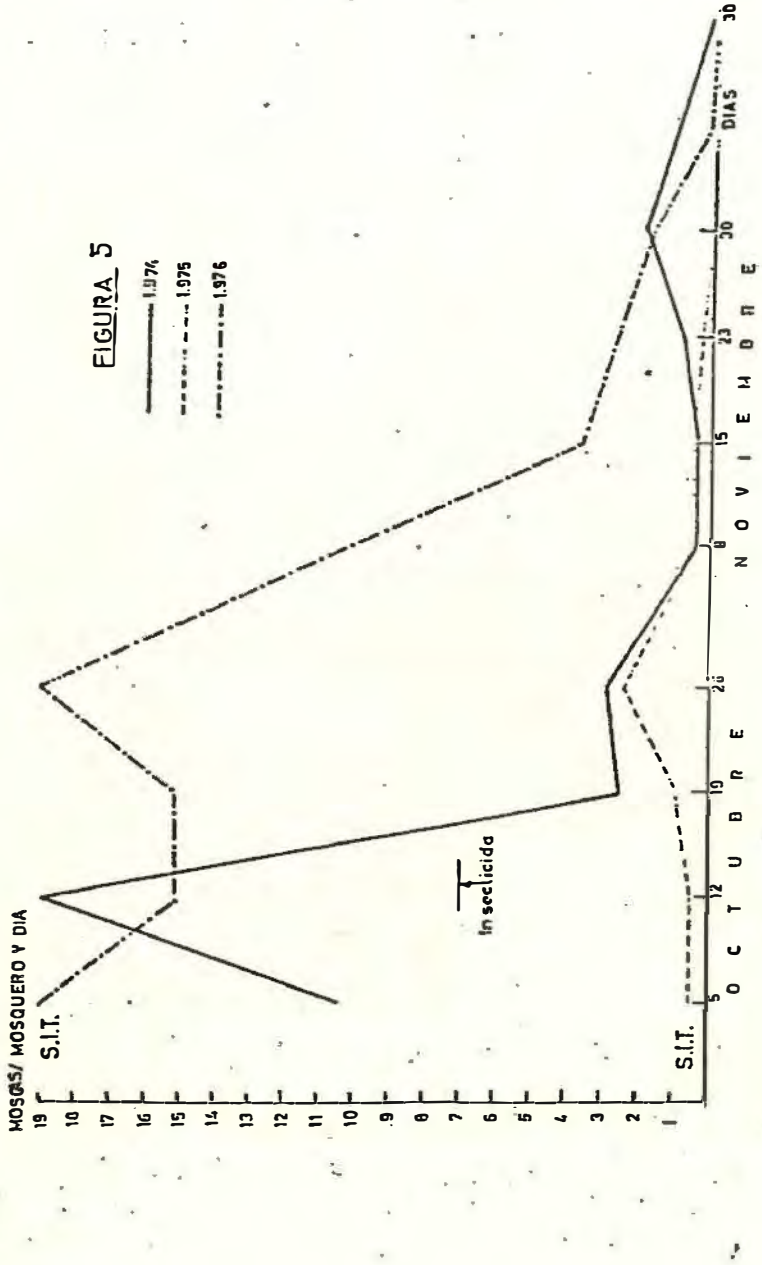


FIGURA 5
1974
1975
1976
MOSCAS/ MOSQUERO Y DIA
S.I.T.
S.I.T.
In seclcida
OCTUBRE
NOVIEMBRE
DIAS

CUADRO - I

CAPTURAS DE CERATITIS EN EL VALLE DE EL GOLFO 1.974 y 1.975

Mes	Día	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Julio	1	2	2	2	9	1	4	4	1	3	1	0	3	3
"	8	0	4	2	0	2	0	3	2	0	0	1	1	1
"	15	1	1	1	2	0	1	2	4	1	0	1	3	1
"	21	0	2	4	9	1	0	0	7	9	1	3	21	4
"	29	1	1	2	2	0	1	1	1	0	1	1	5	1
Agosto	13	3	5	2	10	7	5	4	42	25	4	10	30	11
"	10	4	5	6	12	13	25	10	20	25	50	90	10	12
"	17	12	15	16	12	12	15	10	81	70	47	55	187	54
"	26	9	30	15	38	20	60	32	20	52	131	185	190	80
Septiembre	7	30	50	28	61	93	89	56	150	388	120	163	322	90
"	14	6	13	17	70	50	74	21	111	90	120	83	190	89
"	23	8	10	22	31	63	60	16	100	32	104	90	187	47
Octubre	5	20	26	51	90	66	56	32	215	190	82	62	114	35
"	12	18	23	60	230	201	190	85	310	214	80	62	214	60
"	17	Tratamiento insecticida												
"	19	6	4	12	13	11	17	9	14	10	20	7	32	44
"	26	6	4	9	14	11	22	16	54	38	14	6	20	41
Noviembre	8	2	0	1	4	2	6	3	14	17	0	1	9	7
"	16	0	2	4	0	3	5	0	12	8	0	2	5	0
"	23	2	4	0	6	9	6	8	14	22	0	0	3	0
"	30	0	0	0	6	2	1	4	60	82	5	0	8	0
Diciembre	28	0	0	0	0	0	0	2	6	4	0	-	0	1

CUADRO-II

CAPTURAS DE CERATITIS EN EL VALLE DE EL GOLFO 1975

MES	DIA	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Enero		0	0	0	0	0	0	0	0	0	0	0	0	0
Febrero		0	0	0	0	0	0	0	0	0	0	0	0	0
Marzo		0	0	0	0	0	0	0	0	0	0	0	0	0
Abril	8	0	0	6	0	0	0	0	0	0	0	0	0	0
	11	C O M I E N Z O M E T O D O I N S E C T O S E S T E R I L :												
Mayo		"		"		"			"			"		
Junio		"		"		"			"			"		
Julio		"		"		"			"			"		
Agosto		"		"		"			"			"		
Septiembre	17	F I N A L M E T O D O I N S E C T O S E S T E R I L E S												
Octubre	5	2	0	0	3	0	4	0	6	10	2	0	8	0
	12	3	0	2	1	0	4	6	0	0	12	10	8	0
	19	2	1	0	5	0	3	4	0	1	32	15	21	0
	26	3	0	0	2	1	3	5	0	4	125	34	28	0
Noviembre	8	0	2	3	0	0	1	0	3	4	48	10	12	1
	16	0	0	1	1	0	2	3	4	0	22	5	10	0
	23	1	0	0	0	0	3	1	2	2	8	2	4	0
	30	0	0	0	0	0	0	0	0	0	0	0	0	0
Diciembre	7	0	0	2	0	0	0	0	0	0	0	0	0	2
	14	0	0	1	0	0	0	0	0	0	0	0	1	0
	21	0	0	0	0	0	0	0	0	1	0	0	0	0
	28	0	0	0	0	0	0	0	0	0	0	0	0	0

CUADRO - III

CAPTURAS DE CERATITIS EN EL VALLE DE EL GOLFO 1.976

Fecha	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Enero	0	0	0	0	0	0	0	0	0	0	0	0	0
Febrero	0	0	0	0	0	0	0	0	0	0	0	0	0
Marzo	0	0	0	0	0	0	0	0	0	0	0	0	0
Abril	0	0	0	0	0	0	0	0	0	0	0	0	0
Mayo 15	Se encuentran tres frutos picados												
21	COMIENZO METODO INSECTOS ESTERILES												
Junio	" " " "												
Julio	" " " "												
AGOSTO	" " " "												
Septiembre 23	FINAL METODO INSECTOS ESTERILES												
Octubre 5	34	40	52	12	210	50	29	80	105	290	80	250	120
12	64	45	23	5	230	186	32	94	120	305	93	172	115
19	53	27	36	17	248	105	60	116	95	260	117	185	60
26	40	36	52	10	350	187	71	200	104	290	140	203	98
Noviemb 15	35	28	40	15	100	110	60	90	85	115	60	160	50
Diciemb 15	0	0	0	0	0	0	10	0	2	13	0	1	0

CUADRO - IV

CAMPAÑA CONTRA CERATITIS CAPITATA WIED.- ISLA DEL HIERRO 1.975

CONTROL DE FRUTA

Melocotón

Mes	Días	Kgs.fruta recogidos	% del total de la cosecha	Kgs.fruta atacados	%
Abril	15 - 30	40	0,18	1,2	3
Mayo	1 - 7	270		0	-
	7 - 15	3.600		0	0,02
	15 - 23	1.800		0	-
	23 - 30	3.000		2	0,06
		8.670	39,0	2,75	0,03
Junio	1 - 7	2.500		3	0,12
	7 - 15	4.000		4	0,10
	15 - 23	2.000		3	0,15
	23 - 30	1.000		1	0,10
		9.500	42,8	11	0,12
Julio	1 - 7	1.500		4	0,26
	7 - 15	1.000		5	0,50
	15 - 23	800		10	1,25
	23 - 31	500		15	3
		3.800	17,1	34	0,9
Agosto	1 - 7	100		8	8
	7 - 15	50		10	20
		150	0,6	18	12
	TOTAL	22.160	100	67	0,30

CUADRO-V

CAMPAÑA CONTRA CERATITIS CAPITATA WIED.-ISLA DEL HIERRO 1976

CONTROL DE FRUTA

Melocptón

Mes	Dias	Kgs fruta recogidos	% del total de la cosecha	Kgs fruta atacados	%
Mayo	7-15	20		0,5	2,5
	15-22	50		1,0	2,0
	22-30	100		1,5	1,5
		170	0,9	3,0	1,76
Junio	1-7	80		0,7	0,9
	7-15	200		0,5	0,2
	15-22	1000		2,0	0,2
	22-30	1500		3,0	0,2
		2700	15,3	6,2	0,22
Julio	1-7	2000		7,0	0,3
	7-15	4500		8,0	0,2
	15-22	3000		20,0	0,6
	22-31	2500		14,0	0,6
		12000	66,3	49,0	0,41
Agosto	1-7	1000		20,0	2,0
	7-15	500		15,0	3,0
	15-22	500		20,0	4,0
	22-31	300		20,0	6,6
		2300	12,7	75,0	3,26
Septiembre	1-7	300		25,0	8,3
	7-15	200		20,0	10,0
	15-22	200		25,0	12,5
	22-30	150		20,0	13,3
		850	4,7	90,0	10,59
TOTAL		18100	100	223.2	1.2

CUADRO - VI

CAMPAÑA CONTRA CERATITIS CAPITATA WIED.- ISLA DEL HIERRO 1.977

CONTROL DE FRUTA

Melocotón

Mes	Días	Kgs.fruta recogidos	% del total de la cosecha	Kgs.frute atacados	%
Mayo	7 - 14	500		3	0,6
	15 - 22	3.200		6	0.2
	22 - 29	3.800		11	0.3
		7.500	17,2	20	0.27
Junio	30M - 5J	4.900		5	0,1
	6 - 12	5.200		10	0,2
	13 - 19	3.000		12	0,4
	20 - 30	500		2	0,4
		13.600	31,1	29	0,29
Julio	4 - 10	2.500		15	0,6
	11 - 17	4.000		30	0,75
	18 - 24	5.000		60	1,2
	25 - 31	6.150		70	1.15
		17.650	38.1	175	0.99
Agosto	1 - 7	3.200		96	3
	8 - 15	1.750		146	8.3
		4.950	11.3	242	4,91
	TOTAL	43.700	100	463	1,06

THE INTERNATIONAL SIT FIELD PROGRAM AGAINST *Rhagoletis cerasi* IN SWITZERLAND

E. BOLLER, U. REMUND & B. KATSOYANNOS, Switzerland
K. RUSS, Austria

1. Objectives of the program

Based on the recommendation of the IOBC working group on genetic control of *Rhagoletis cerasi* at the last meeting in 1975 the Swiss Federal Research Station at Wädenswil and the local fruit growers in northwest Switzerland decided to initiate a 4 year field demonstration of the SIT to start in 1976.

The objectives of the operation involving 1400 cherry trees, dispersed over a 2.5 km2 surface area and relatively well isolated by forests, were defined as follows :

a) Elimination of the pest species in a larger target area between 1976 and 1979
b) Establishment of a cost-benefit analysis in 1979
c) Development and application of the technologies involved and implementation of a strict quarantine program by establishing effective buffer zones
d) Final report and recommendations with regard to the feasibility of future applications of the SIT in selected target areas.

2. Materials and methods

The flies required for this field program were produced in field collection centers operated in Switzerland and Austria that provided the necessary amount of diapausing pupae. These pupae were reared from highly infested cherries bought in bulk and spread over ca. 1000 m2 of screens placed over vermiculite where the extracted larvae pupated. After 5 days the cherries were removed from the screens and sold to distillation plants for final utilization. In addition to cherries infested *Lonicera* berries where harvested in bulk along the super-highways providing a free and yielding source of *Rhagoletis* material. After chilling the pupae for 5-6 months they were incubated according to the established release schedule. The flies were marked with fluorescent KRYLON spray soon after emergence, fed an internal marker (dysprosium chloride) for 2 days, irradiated at the age of 2 days with 9 krad and released on the 3rd day. Releases were carried out 3 times a week beginning 5 weeks before harvest and continued up to harvest time of the middle cherry varieties.

A laboratory rearing program was providing additional fruit fly material and a comprehensive quality control program was implemented to monitor the flies' performance.

3. The release operation

The target area is subdivided into 3 subareas I, II and III by forest and fields containing no host trees. Subareas I and II (containing some 500 trees) were treated in 1976 with sterile flies. That release program continues until 1979. Subarea III with 875 trees had an unknown history of infestation because most of the trees were not treated with insecticides. Therefore, we decided to suppress the population in 1976 with a mass-trapping program (ca. 3500 Prokobol-traps) and to initiate the sterile fly release in 1977. From 1977 to 1979 the entire complex is included in the SIT program.

4. Results and discussion

The most relevant data are given in Tab. 1 and Fig. 1. 1976 : The impact of the sterile flies in 1976 was evident at harvest time as infestations in the release areas dropped below detectable levels. The suppression program with traps was also a full success as most cherries were harvested without infestation except on trees situated in a hot spot (maximum infestation observed was 6 %). This encouraging result achieved in the first year was significantly caused by the high cherry yield that is an important prerequisite for the success of a genetic control program. All observations made during the release period indicated that the sterile flies performed well and did not move out of the release areas.

1977 : That year was characterized by most difficult conditions. Late frosts destroyed most of the cherries introducing unpredictable elements with regard to the dispersive behavior of the sterile and wild flies. In addition to these environmental difficulties the amount of pupae obtained in 1976 in the field collections did not reach the anticipated number of 6-700'000 pupae needed to cover the 1400 trees now included in the release schedule. Only 400'000 flies were available and released in such a way that the most critical stages of the cherries could be covered with sterile flies. As shown in the table and the figure this objective could be fulfilled for the middle cherry varieties that could be harvested in most cases without infestation (with the exception of a few trees that showed maximum infestation levels between 4 and 8 %). The average infestation in the low yield of middle late varieties in the 3 subareas was 0, 0.6 and 0.8 %, respectively. Under normal crop conditions these figures would have dropped below detectable levels.

The problems occurred during the period where the very susceptible late varieties were in prime stage for oviposition (last week of June - first week of July). The number of wild flies started to increase in a pattern that did not reflect the normal flight characteristics observed in comparable check areas. Dispersive movement was increasing as soon as the middle varieties had passed the optimal stage for attack when the flies were searching for late varieties. Consequently the over-

flooding ratio on late cherry trees was decreasing constantly and fell below the critical 20 : 1 level towards the end of the release period end June. Early warning signals were picked up when egg collections made June 24 showed for the first time an increase in the fertility level (Fig. 1, top). This situation became more severe early July when a sudden increase of wild flies was observed that coincided with the beginning of cherry harvest. We cannot exclude the hypothesis that these wild flies immigrated from a heavily infested cherry plantation situated close to and not well isolated from area III.

5. The situation in 1978 : Conclusions

Based on the observed difficulties encountered during the most difficult year 1977 we have taken the necessary steps to solve the existing problems.

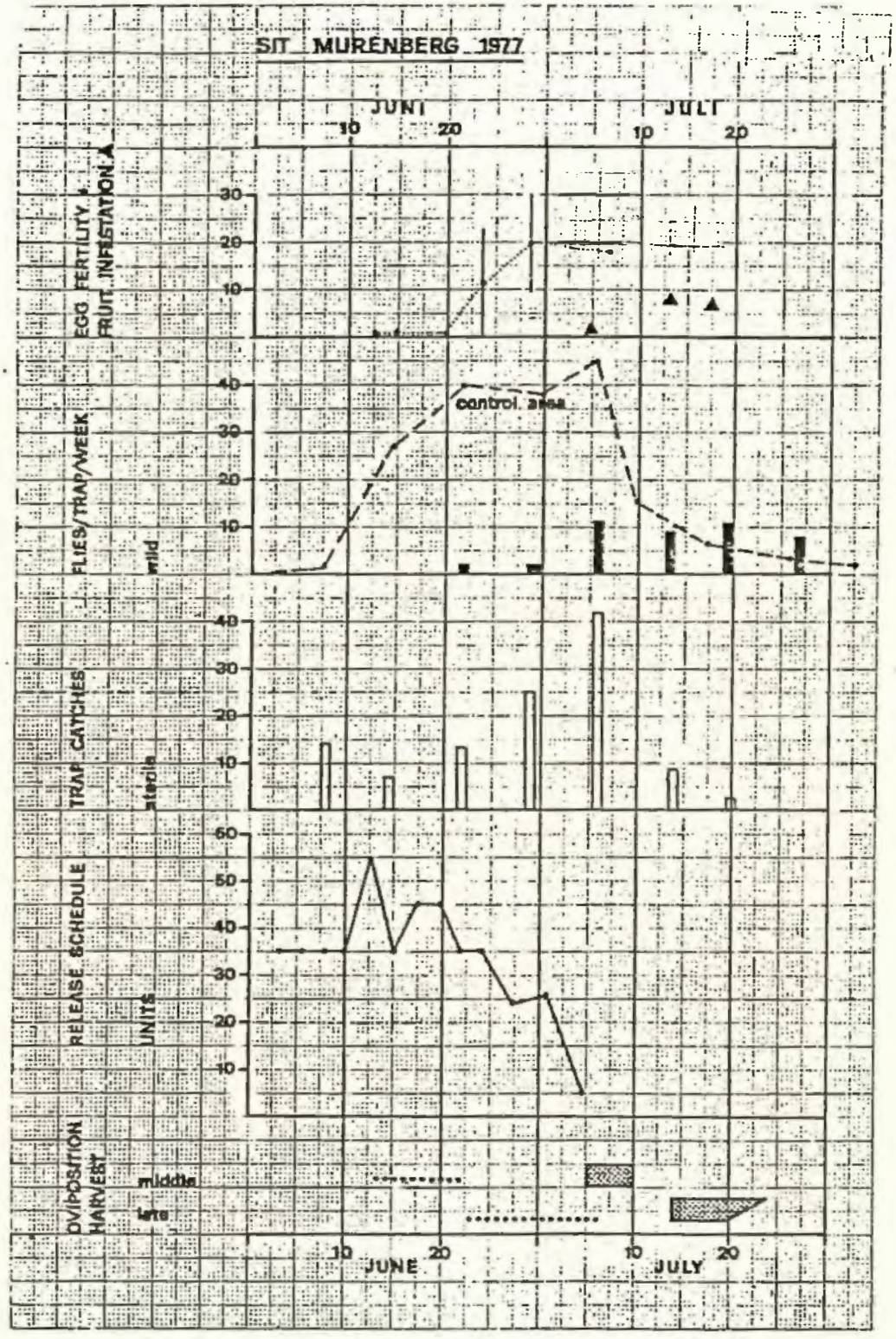
The number of flies available for 1978 is about 1'000'000- a significant increase of the impact be expected this year. The buffer zones around the release areas will receive special attention. Adjacent orchards that had not been sprayed last year were treated in March 1978 with efficient insecticides applied to the soil underneath cherry trees to destroy the hibernating pupae. All orchards within a 1-km range around the SIT area will receive insecticide treatments against the cherry fruit fly in order to minimize the danger of immigration in case the cherry yield should again be low due to late frosts.

A dense trap-network will be operated outside the target area in order to monitor the populations in the buffer zones. Special attention will be given to the aspect of long range dispersal of *Rhagoletis cerasi* that was never considered to be a major problem but could be a potential hazard to SIT programs.

In conclusion we can say that the present status of the SIT against *Rhagoletis cerasi* justifies an optimistic outlook. The technologies involved have reached a stage where larger programs could be carried out. The potential limiting factors - that are still under careful investigation - are the problems of implementing effective quarantine (including buffer zones) in order to keep the pest outside the target area, the question for what period of time the treated area can be kept clean and as a consequence of this how the cost of a genetic control program compare with the traditional chemical control method. In case we achieve complete elimination of the pest by 1979 and can keep it out of the area for a number of years - then also the economics will reach a level where SIT could become an interesting alternative to present pest control strategies.

Table 1 : Parameters of SIT program in Northwest Switzerland (Rhagoletis cerasi)

Year	Subarea	No of trees	Crop	Treatment	Sterile flies per tree	Average ratio	Infestation at harvest % Average	Range
1975	I	330	high	Insecticide	-	-	0	
	II	184	high	Insecticide	-	-	0	
	III	875	high	none	-	-	?	
1976	I	330	high	Sterile flies	245	29:1	0	
	II	184	high	Sterile flies	375	27:1	0	
	III	875	high	Traps (4.3/tree)	-	-	0.3	0 - 6
1977	I	330	low	Sterile flies	250	20:1	0 (middle)	0
						1:1	1.4 (late)	0 - 9
	II	184	low	Sterile flies	600	30:1	0.6 (middle)	0 - 4
						6:1	2.8 (late)	0 - 10
	III	875	low	Sterile flies	240	34:1	0.8 (middle)	0 - 8
						2:1	8.7 (late)	0 - 28
	check area		low	none			40 - 50	
1978	I	330	?	Sterile flies	720			
	II	184	?	Sterile flies	720			
	III	875	?	Sterile flies	720			


SIT MURENBERG 1977
JUNI
JULI
10
20
10
20
EGG FERTILITY
FRUIT INFESTATION
30
20
10
FLIES/TRAP/WEEK
40
30
20
10
control area
wild
TRAP CATCHES
sterile
40
30
20
10
RELEASE SCHEDULE
UNITS
60
50
40
30
20
10
OVIPOSITION
HARVEST
middle
late
10
JUNE
20
10
JULY
20

THE POSSIBLE USE OF IIT TO CONTROL *Rhagoletis cerasi* L., THE EUROPEAN CHERRY FRUIT FLY IN AUSTRIA

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Within the framework of the IOBC/WPRS working group "Genetic control of European cherry fruit fly" F. BOLLER (1974) demonstrated for the first time the existence of at least two unidirectional incompatible races of the European cherry fruit fly. Since then numerous single investigations and crossing experiments involving flies from nearly all areas of Europe made it possible to obtain a very good survey of the racial distribution and the borderline between the two races.

The facts that this border-line (or border-area) runs through Austria (northern Lower Austria), and that crossing-experiments showed a sterility of produced eggs when males from southern areas were crossed with females from northern areas, were occasion for us to try to use this biological idiosyncrasy of the cherry fruit fly in a new type of pest management method.

Experiments in 1977

In 1977 the first small-scale realease experiments in the field were carried out on a small number of cherry trees in the distribution area of the northern race, whereby approximativ 50.000 adult males (southern race) of the cherry fruit fly were released.

Unfortunately these first experiments brought no results, because intensive frost damage to the cherry trees prevented exact execution of the experiments. But it was possible to obtain diverse methodical results pertaining to these experiments. Especially the transport of adult male flies the approximative hundred kilometers to the experimental area resulted in relatively high losses. Our marking method (use of spray paint) was also difficult to manipulate and probably also reduced the number of flies even before and after the release. Very serious however was the circumstance that, because the cherries (medium late typus) were frozen off, there developed an extraordinarily high population density on the few cherry fruits on the experimental trees. Therefore the relatively small number of released male flies were largely excluded as copulatory competitors by the native population.

Experiments in 1978

A relatively large release experiment involving approximative 200.000 males of the "southern race" from Burgenland and Switzerland in an experimental area containing 120 cherry trees in the northern distribution area is planned for 1978.

In contrast to 1977 no adult males will be released in 1978 but rather male pupae using a specially designed program. The determination of the male pupae is conducted by using a special "pupae-sorting maschine" designed with the cooperation of the laboratory of E. BOLLER (Wädenswil) by the Austrian Plant Protection Institut (ZELGER, RUSS, 1976). In order to depress the natural fly population and consequently to increase the effectivity of the released males the soil of the experimental area was treated with Basudin (0,3 % , 1 l/m2).

The necessary pest management method planned for 1978 by using IIT (incompatible insect technique), the release method as well as other experimental details will be described during the meeting.

1. INFLUENCE OF THE SEX PHEROMONE ON TRAP EFFICIENCY AND SEX RATIO (R. cerasi L.)

typ of trap	n	flies per trap	rel. effectivness	sex ratio ♀♀:♂♂	year
PROKOBOL II (control)	5	6.6 a[3]	1.00	0.57 : 1	76
PROKOBOL II + 20 virgin ♂♂	5	23.0 b	7.00 (3.8 x ♀♀, 3.2 x ♂♂)	0.67 : 1	
REBELL (control)	10	27 a	1.00	0.81 : 1	77
REBELL +100 calibr. ♂♂ pupae[1]	10	28 a	1.03	1.72 : 1	
REBELL +50 virgin ♂♂[2]	10	43 b	1.59	1.39 : 1	

1 over 90% of males
2 fresh ♂♂ after 2 weeks
3 multiple range test

2. INFLUENCE OF AN ATTRACTANT AND TRAP CROSSING

typ of trap	PROKOBOL II		REBELL 77	
	single trap	crossed trap	single trap	crossed trap
with attractant[1]	1.22	2.39	2.18	2.38
without attractant	1.00	1.88	1.10	2.29
sex ratio	0.74 : 1		0.93 : 1	

1 10 g Ammonium acetate per trap

Comparison and application of traps for Rhagoletis cerasi L.

U. Remund and E. Boller, Wädenswil, Switzerland

1. Comparison of fluorescent and not-fluorescent yellow types — July 7 to July 18, 1977

type of trap	number of replications	flies per trap	relative effectiveness	sex ratio ♀♀ : ♂♂
Prokobol II	10	7,43 a	1,00	0,52 : 1
Rebell, 3% N.B. 103	10	48,17 b	6,15	1,78 : 1
Rebell, 0,1% N.B. 103 and 3% S.Y.	10	21,66 b	2,92	2,10 : 1
Rebell, 3% N.B. 2801	10	32,83 b	4,42	1,70 : 1
Rebell, 0,1% N.B. 2801 and 3% S.Y.	10	34,83 b	4,69	1,71 : 1
Rebell, 3% N.B. 2751*	10	60,67 c	7,75	1,15 : 1
Rebell, 0,1% N.B. 2751 and 3% S.Y.	10	38,67 b	5,52	1,52 : 1

* Rebell 78

Means followed by the same letter are not significantly different according to Wilcoxon and Wilcox's multiple range test (p=0,05). N.B. = not fluorescent colors; S.Y. = fluorescent colors.

2. Application of traps for control purposes

Orchard	number of trees		number of traps per tree	flies per trap	Portion of trees without infestation
	treated	checked			
Nurenberg 1976	700	44	4.3	2.34	90.9%

3. Application of traps for prevision purposes

Orchard and year *	number of flies per trap 3 weeks before harvest	number of flies per trap at harvest	chemical treatment	infestation in %
Wangen 1976	0	0	none	0
Wangen 1977	0	1,3	none	0
Wangen 1978	?	?	?	?

* 5 Rebell traps distributed on 120 trees (closed plantation)/year

Application of insecticides in the years before 1976 (very low population)

PROSPECTS FOR THE CONTROL OF *Dacus oleae* (GMELIN) (DIPTERA, TEPHRITIDAE) BY METHODS THAT DO NOT INVOLVE INSECTICIDES. THE STERILE INSECT RELEASE TECHNIQUE AND OLFACTORY AND VISUAL TRAPS. INTEGRATED APPROACH.

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Abstract

Laboratory and field experiments showed that the sterile insect release technique can reduce the olive fly damage to the crop in isolated olive groves. More research and field applications are still needed to simplify the method and make it cheap and practical.

The fluorescent yellow color was found to attract more olive flies than any other color. Fluorescent yellow panels covered by sticky material were successfully used to control the olive fly in a small olive grove. Again, more research is needed to make the method practical and study its effect on the ecosystem.

McPhail odor traps covered by sticky material proved more powerful than the fluorescent yellow traps against *D. oleae*. They attracted the fly from longer distances than the color traps which are usually effective only within the tree they are hung. The finding that sexual pheromones are involved in male attraction suggests that the chemicals involved could be used (when identified and synthesized) to trap or confuse the olive fly in the field.

The sterile insect technique could be combined with parasites for the immature stages. The latter could also be combined with color traps provided that they are not attracted by fluorescent yellow. It is believed that the above methods (when perfected) could be applied in a combination or succession around the year which will effectively protect the olive crop against *D. oleae*.

Introduction

The olive fruit fly, *Dacus oleae* (Gmelin), is considered the most serious olive pest in many of the olive growing countries of the Mediterranean basin. In Greece, for example, 225.000 tons of oil and 55.000 tons of table olives of an approximate value of 370 million U.S. dollars were produced in 1976. If no insecticides were used, the damage could go up to 30-40 % or even higher. The use of country-wide applications of insecticides kept the damage to levels below 5 %. Cover or

bait sprays with organophosphates are usually applied several times from June-November. For 1976, the cost for controlling *D. oleae* throughout Greece was estimated to ca. 9 million U.S. dollars. It is true that for many years the fly has been effectively controlled by wide use of synthetic insecticides (often applied by airplane) which have also produced extensive poisoning of other organisms in the olive-tree agroecosystem. Thus, the need for a control system that will keep the damage below economic levels and will not harm the environment is deemed necessary.

A Sterile Insect Technique (SIT) panel for the control of fruit flies, held by FAO/IAEA in Vienna in 1973 made the following recomendations on *D. oleae* : "The SIT can and should fit into a pest management system for olive fly control. Because of a lack of geographical isolation, eradication usually will not be the objective of the SIT in olive fly suppression programmes. The pest management scheme should comprise two sequential phases :

a) suppression : by cultural practices (spring collection of remaining fruits), treatment with selective insecticides (early summer), trapping (pheromones included) ;

b) control : by release of sterile olive flies in combination with parasites (1).

Insecticides and biological control against *D. oleae* have been studied since many years. Recently, much research is also devoted to trapping systems and the SIT visual traps and the SIT in combination with insecticides have been tested for control purposes in the field with promising results. In this paper the SIT and trapping systems are discussed to some detail. Both appear promising and their potential role in an integrated control system is being studied during the last years.

Sterile insect technique

It has been reported that when a chemosterilant (Apholate) was made available to the olive fly through McPhail olfactory traps the result was to produce substantial decline in the reproduction of the population in an olive grove of ca. 2000 trees (2). Lab experiments have also shown that other chemosterilants could also sterilize the olive fly effectively. Nevertheless, no much effort has been invested so far on this technique, that is to sterilize the wild population, because of the risk to contaminate the environment with highly dangerous substances. On the other hand the attention has concentrated on the sterile insect release technique which involves artificial rearing of the fly, sterilizing it and subsequently releasing in the field. Of course, this approach makes the technique more complicated and difficult since additional problems, e.g. artificial rearing, quality, release techniques, must be solved.

a) Artificial rearing

After several years of research an efficient system for rearing the fly in big numbers has been developed. Currently, the production cost is ca. 1 U.S. dollar per 2000 insects. Large cages, 100 cm L x 40 cm D x 30 cm H, are used, in which the fly density can be 1800-2400 flies per cage. The flies oviposit in cone-shape oviposition substrates made of nylon gauze coated in ceresin. The larval diet contains : tap water 55 ml, cellulose powder 30 g, brewer's yeast 7.5 g, soy hydrolyzate enzymatic 3 g, brewer's yeast 7.5g, sucrose 2 g, olive oil 2 ml, Tween-80 0.75 ml, potassium sorbate 0.05 g, Nipagin 0.2 g, and HCl 3 ml (3,4).

b) Sterilization

Artificially reared flies are sterilized in a CO^{60} γ-ray source. Pupae are treated one day before emergence in nitrogen atmosphere at 11 krad. This method produces more competitive insects than irradiating pupae or adults in air (5). It has also the advantage that the females do not produce many oviposition holes (since they have no eggs due to pupal irradiation) and this prevents secondary fungi infestations (6). The latter is important in humid areas.

c) Quality of insects to be released

Besides mass rearing and sterilization, a successful application of the sterile insect release technique also necessitates high quality of released insects. It has been a matter of growing concern in recent years that the simplification and automation of the rearing procedures deteriorates the insect quality. Sterilization also affects the vigor and competitiveness of the insect.

In *Dacus oleae*, artificial rearing has produced insects selected for high egg production in the 1st weeks of their life (7), and earlier sexual maturation in both sexes as compared to wild flies (ZERVAS, unpublished data). Sperm depletion in artificially reared females was also found to occur sooner than in females reared on olives (in both cases females were mated with same type males) (8). Although irradiation affects the survival of flies and their mating competitiveness (9, 5), a 2d mating by a sterilized male was found to reduce effectively fertile reproduction in both artificially reared or reared on olives *D. oleae* unless the sterilized male had already depleted its sperm through repetitive matings (10, 11). Overcrowding during the larval stage of artificially reared flies was found to affect negatively adult weight, survival, reproductive potential and male competitiveness (12). Finally, both artificial rearing and γ-irradiation were found to improve pheromonal attraction of males by females (HANIOTAKIS, unpublished data).

Under field conditions, the responses of artificially reared and wild flies to host plant color and odor, host fruit color and shape, small rectangles of different colors and shades, and McPhail-type traps of different colors baited with different odors were compared. Qualitatively, the responses of the two fly types toward the various experimental treatments were essentially the same, except for the artificially reared flies being relatively more attracted toward red color and relatively less attracted toward yellow color, than the wild flies. Quantitatively, however, the data suggested that the mobility, flight pattern, or vigor of the two types of flies may be different (13). In other studies, artificially reared flies were found to possess inferior dispersal ability than wild flies (14, 15). Irradiation had no apparent effect on dispersal (16). Finally, no differences were found in the ability of either flies from northern and southern Greece, or normal and γ-irradiated artificially reared flies to acclimate to winter conditions in the field (17).

d) Field application

A combination of insecticides at the beginning of the summer and weekly releases of sterilized flies thereafter was applied for two consecutive years in a semi-isolated olive grove of ca. 600 trees in northern Greece (6). Releases started before fruits became suitable for oviposition, two weeks after the application of insecticides. Sterilized *D. oleae* were placed into expanded paperbags. For release, the bags were torn open and hung on release poles places equidistantly among 4 olive trees. Between release poles the distance was ca. 60 m. In each release, ca. 150,000 to 200,000 flies of both sexes were released, resulting in high ratio of sterilized to wild flies. In the 1st year flies were irradiated at the adult stage while in the 2d at the pupal stage in nitrogen.

In the 1st year fruit infestation in the release grove never rose above 10 %, while in the control groves it was much higher. A similar trend was followed in the 2d year although absolute values were much higher. The weather was very favorable for *D. oleae* development in the 2d year, and this resulted in nigh wild population in the area. Thus, although fruit infestation was kept low in the release grove till the beginning of October, it rose quickly thereafter probably due to wild flies immigrating from neighboring areas. In the control groves infestation was very high from the beginning of September. The conclusions from the two-year application were :

a) Releases of sterile insects can considerably reduce olive fruit fly infestation

b) The timing of the various treatments (insecticide applications, releases of sterile insects) depends mainly on weather, crop maturation, and olive fly population and its reproductive potential.

c) The method should be applied simoultaneously in continuous olive groves which must be well isolated to avoid immigration of flies from other olive growing areas.

Trapping methods

One of the control methods that harm very little the environment is to lure the insect-pest to devices which kill it or make it unable to continue normal feeding and reproduction in nature. The impact of such methods to beneficial insects should be of course investigated. Against *D. oleae*, both olfactory and visual lures have been studied.

a) Olfactory lures.

The McPhail glass trap baited with ammonium salts or various odor solutions (commonly called "protein hydrolyzates") has been used for wild population monitoring in spray programs for many years. Recently, it was found that when the exterior surface of the trap was covered by sticky material its efficiency was increased by 2 or 3 times since all arriving flies were catched (otherwise many of them would fly away before drown into the lure solution) (18). Thus, as of this moment, the most powerful olfactory trap against *D. oleae* is considered the McPhail glass trap baited with : "protein hydrolyzate" (Rodia TM or Zitan-98TM or others) + borax water solution + sticky material on the exterior. This trap was recently compared to fluorescent yellow color trap in the field and proved much more powerful, especially in attracting *D. oleae* from distances longer than the diameter of the tree where the trap is hung (ECONOMOPOULOS, unpublished data).

Sexual pheromones have also attracted the interest of researchers. Both sexes of *D. oleae* heve been reported to emit characteristic odors during the mating hours (19). Mature virgin females were subsequently found to attract sexually the males in the field (20). Volatile substances collected from laboratory and wild female *D. oleae* were also found to attract males of the same species during the hours of sexual activity (21). The presence of an airborne sex pheromone was thus verified. Current research aims to identification and synthesis of the chemical substance(s) involved. These substances could eventually be used in the field for control purposes (attraction to traps or confusion and disruption of normal sexual behavior).

b) Visual lures

In recent years, color response has been used to develop traps against several fruit flies. Such traps have been used against the olive fly in Israel (22) and in Italy (23). The fluorescent yellow color was found to attract more *D. oleae* than any other color. The response of the fly was found to be positive attraction to the color and primarily to the hue and not the intensity. The fly was found to be particularly attracted to yellow colors that reflect highest amounts of light between 520-580 nm and little below 520 nm (24). When the fluorescent yellow color was combined with sticky coated McPhail traps baited with Rodia TM , no increase in trap catches

was observed. When the odor lure in the trap was ammonium sulfate or just water, the addition of fluorescent yellow resulted in higher catches (18). Finally, when 3 fluorescent yellow traps were suspended per tree from July till November in an olive grove near Athens, an average of ca. 430 olive flies were trapped in each tree and crop infestation did not increase beyond 15 % as compared to 50 % in the control grove (25). Future work on this subject should include studies on the effect of the traps to beneficial insects.

Future work

Research on mass rearing, quality of flies produced, and field applications should be continued and expanded. It appears that the main obstacle for application over large areas is the rearing cost (facilities, labor, materials). The sterile insect release technique should be repeated in small areas, and as soon as mass rearing becomes easy and cheap, application on large areas should be undertaken. Application of sterile insects in combination with parasites (against the immature stages) should also be tested in the field. The necessity of insecticide applications before start releasing sterile insects should be examined, and if possible they should be kept out of the method. Finally, the role of visual and olfactory traps (pheromones included) in an integrated control system should be investigated. Trapping devices could be used as barriers, especially in narrow valleys, or to monitor the wild population. Of course, in case they prove powerful and practical they could be used as separate control methods.

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PRESENT STATUS OF THE STERILE INSECT RELEASE METHOD AGAINST *Dacus cucurbitae* IN JAPAN

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The first application of the SIRM in Japan was commenced in 1972 to eradicate the melon fly, *Dacus curcurbitae*, from the island of Kume, Okinawa 58.5 sq. km in area. We developed some methods to evaluate the quality of released melon fly. We reports here on the results of our experiment on Kume Is.

The first slide shows the map of the southwestern islands of Japan. When this campaign was planned, Kume Is. was the northern limit of the distribution of this fly in Japan. Unfortunately, adults of this fly were discovered in Okinawa Is. just before the beginning of this campaign. Thereafter the distribution was expanded successively, and this fly reached to the Tokara Islands, near the Main Land, up to 1976.

We established a mass rearing facility on Ishigaki Is. and an irradiation facility on Naha. Naha area was also used as untreated check area of this experiment. The target island of Kume is here. Since 1970 we have set 50 monitor traps baited with cue-lure and naled on Kume Is. these traps were checked bi-monthly. Then we have date on the seasonal changes in relative abundance of the melon fly population on Kume Is. But these data do not provide "absolute" population density from which we should decide the number of sterile flies to be released. Only a method that can be used to estimate the absolute density may be the mark-release-recapture method. Thereby, first of all, we tried to estimate the wild population density of melon fly by this method. For this purpose we developed a new mathematical model which based on some modifications of Jadkson's positive method (ITO, 1973). We'll omit the explanation of equations to save our talking time.

Based on data obtained by the marking method and informations on the relative population level in different vegetations, the number of sexually matured wild males on Kume Is. was estimated to be about two millions and a half individuals in the peak season. On the other hand the capacity of our mass-rearing facility was, at first, one million pupae per week.

Before the beginning of the mass-release on Kume, we conducted a pilot release experiment on a small islet of Kudaka, near Okinawa Island from November, 1974. The number of adult flies of both sexes on this islet was estimated to be ca. 10,000 and we released 100 thousands sterile pupae per week (IWAHASHI, 1976). Irradiation was made on two days before adult eclosion at a dose of 7 KR. This dose resulted in complete sterility of females and almost complete sterility of males.

This second slide is a part of the results of the pilot release experiment on Kudaka Is (table 1). The number of marked and unmarked males caught in monitor traps, and the percent hatch of eggs collected from fuits on Kudaka Is. and control area where no release was made, are shown. By using Fried's equation (FRIED, 1971) we could calculate the index of sexual competitiveness, C, of sterile males under field conditions. That is

$$C = \frac{H_n - H_c}{H_c - H_s} \cdot \frac{N}{S}$$

Here H_n is the percent egg-hatch in matings between normal males and normal females, H_s is the percent egg-hatch in matings between sterile males and normal females, H_c is the percent egg-hatch in competitive matings at a given ratio of sterile to normal males and S and N are the number of sterile males and normal males, respectively. H_s is zero in our case.
Substituting values of percent egg-hatch on Kudaka Is. for H_c, those at control area for H_n, the ratio of marked flies to normal ones in monitor traps in this equation, C could be obtained as shown in the table. The mean value of $\bar{C}$ was estimated to be 0.75. This value is not different from the value that obtained in laboratory experiment. Thus, in our melon fly, reduction of sexual competitiveness was not so serious as compared with some other fruit flies.

Judging from the result that the percent egg-hatch decreased as the ratio of S/N rose on Kudaka Is., it was suggested that the SIRM could be the available technique to control the melon fly. Thus we made bold to start mass release of sterile flies against Kume Is. After reducing the population density of the melon fly with distribution of poisoned cue-lure strings and protein bait sparays, we started weekly releases of sterile flies on Kume Is. in February, 1975. Fly pupae reared in the mass rearing facility on Ishigaki Is. were transported by air and irradiated at a dose of 7 KR, two or three days before adult eclosion. After irradiation, the pupae were transported again by air to Kuem Is., marked with a fluorescent dye, and then released using specially designed release buckets.

This third slide shows an irradiation equipment with three turn-tables. One million pupae could be sterilized for 30 minutes of one irradiation treatment.

Initially we released one million pupae per week from 170 points (fig. 2). The left shows release points and the right shows the distribution of 50 monitor traps baited with cue-lure and naled. To evaluate the ratio of sterile to wild flies, flies caught in these traps were removed twice a month and heads of all the flies were crashed individually on a filter paper, and filter papers were examined under an ultra-

violet lamp for fluorescent marks. Also we examined the rate of egg-hatch for the target and the control area. Female flies were collected by a net from cucutbit fields, and brought to the laboratory. Then these flies were reared individually in plastic containers and eggs were collected with an artificial oviposition device.

Open circles of the top of figure 3 indicate the number of sterilized pupae released per week. Solid circles are the number of adults emerged, which was estimated from the rate of emergence of pupae in sampled release buckets. Middle graph shows the change in the ratio of marked to unmarked flies, M/U, caught in monitor traps. Bottom graph shows the trend in hatchability of eggs laid by female collected on Kume Is. and Naha, the control area.

The ratio of M/U exceeded unity in March, 1975. The ratio decreased thereafter inspite of the increased numbers of sterile flies released. One reason for this was probably due to a seasonal rise in the population density of wild flies which exceeded the increase in number of released flies. Another reason might have been due to an underestimation of the ratio, because since June, determination of marked flies with an ultraviolet lamp became difficult due to the frequent occurrence of a natural fluorescence. This natural fluorescence was considered to be due to decomposition of dead flies in traps during warm and humid conditions. Thus, since June, only those flies with an unmistakable fluorescence were counted as marked flies and flies having a natural fluorescence were counted as unmarked flies. Thus the ratio of M/U might be underestimated since June. The high mortality rate of released pupae was also considered to be the cause of the decrease in the M/U ratio. However, we feel that the primary reason fot the low M/U ratio was the small number of flies in this stage. Thus it was reasonable that the percent egg-hatch on Kume Is. in July was not different from that of the control area.

After September, 1975 we increased the number of released pupae up to two millions per week. Consequently the M/U ratio become increased since autumn of 1975. Also the percent egg-hatch on Kume Is. consistently decreased from August onward and reached 45 % in November, 1976. These results suggested that the SIRM was playing their role to control the melon fly population on Kume Is., but the number of flies released was considered to be insufficient to lead the eradication of this species within a short time.

By the way, we said that determination of marked flies with an ultralamp was difficult since June, 1975. For this reason the M/U ratio might be understimated. Then we attempted to estimate the ratio of sterile to normal flies by using the other methods.

This is Fried's equation, which was originally proposed to estimate the sexual competitiveness of sterile flies. We can modify the equation to give :

$$\frac{S}{N} = \frac{H_n - H_c}{H_s} \cdot \frac{1}{C}$$

If we obtain data on H_n , H_c and C, we can estimate the ratio of S/N from this equation. We used the percent egg-hatch in Naha, where no release was made, as H_n and that on Kume Is. as H_c , C was tentatively assumed to be a constant value of 0.75 that was obtained from the pilot experiment on Kudaka Is. As the irradiation of 7 KR results in almost complete sterility, we can cancel H_s out.

Meanwhile female melon flies irradiated at 7 KR oviposite no eggs. I said that we recorded the rate of egg-hatch for individual female. Then, we can estimate the ratio of the sterile to normal females, S/N, from oviposition data. That is,

$$\frac{S_f}{N_f} = \frac{T_f - RT_o}{RT_o} \quad \text{and} \quad R = C_f \ / \ C_o$$

Here T_f is the number of females collected on Kume Is., T_o is the number of females on Kume Is. and which oviposited eggs. R is the reciprocal of the rate of oviposited females in the control area. Then denominator, RT_o indicates the number of wild females within a total number of females collected on Kumes Is., and numerator indicates the number of sterile females.

When the actual ratios of sterile to normal flies are not different between sexes, S_f / N_f can be directly compared with S/N.

Open circles indicate S/N, solid triangles indicate S_f/N_f and solid circles indicate M/U (fig. 4). From this figure we can see that S/N are nearly equal to S_f/N_f , but higher than M/U in warm season of 1975. Then, we can consider that S/N might be a precise index of the ratio of sterile to normal flies on Kume Is. Thus the values of M/U probably understimated during warm season in 1975. These three values agreed well in March and May, 1976, when the natural fluorescence disappeared. Good agreement of the values of S/N which include C and S_f/N_f which don't inclucle C, suggests that there was no serious reduction of the sexual competitiveness of released flies under natural conditions of Kume Is.

Apart from the studies on the estimation of the sterile to normale flies. We like to speak again the results of the SIRM on Kume Is. Although the SIRM was playing their role to control the melon fly after autumn in 1975, but the number of

released flies were considered to be insufficient to eradicate within a short time. The question was how many flies we should release to get an eradication.

ITO (1977) made a simulation of the SIRM process using population parameters obtained during the course of our project. This suggests that an increase of sterilized flies released up to 4 million pupae per week can speed up the eradication process on Kume Is. This was realized since May, 1976.

The ratio of M/U exceeded 2 in February, 1976. After that this value has increased successively and reached to 90 in September.

The rate of egg-hatch decreased to 29 % in March and a further decrease to 20 % was recorded in May. This value dropped to 1.5 % in July. On the other hand, the rate of egg-hatch in the untreated area ranged 72 to 78 % during the same period.

Fig. 5 shows "female" melon fly catches in monitor traps per 10,000 male fly catches in monitor traps during April, 1976 to March, 1977. Usually the catches of female flies in cue-lure traps were rare. However this value began to increase from August, 1976. This might be a sign that the wild melon flies on Kume Is. became to nearly extinct in August, 1976.

The decrease of wild fly population was also suggested from data on fruits examination (fig. 6).

We collected more than 5,000 fruits of a wild cucurbit plant, *Bryonopsis laciniosa*, per month and checked the infestation by the melon fly. This slide shows the trend of percent infestation of fruits. The mean percentage of infested fruits in the pre-release period ranged from 2.6 to 23.6. During the first year of the SIRM program, there was no sign of remarkable decrease in the percent infestation. After May, 1976, however, it decreased rapidly. From October, 1976 to September, 1977 more than 150 thousand fruits of host plants, mainly *Bryonopsis laciniosa*, were gathered on Kume Is. and examined in Naha. But, there was no sign of melon fly infestation during these twelve months. Thus we concluded that the eradication of the melon fly population on Kume Is. with the SIRM was achieved.

The first reason that we could obtain a success in the application of the SIRM to eradicate the melon fly was that we evaluated accurately the process of our Kume project with elaborate field surveies. The second reason was that there was no serious reduction of the sexual competitiveness of the released flies under the natural conditions of Kume Is.

After the Kume project we are now releasing sterile adult flies from a helicopter to islets near Kume Is. Releases to these islets will be continue to the time when a large scale release project, may be 100 million flies or more per week, which covers all the parts of Okinawa Is. and Amami Is. shall begin. To realize this large scale project we have begun to design a new large-scale production facility.

Table I Estmation of sexual competitiveness, C, of sterile males of *Dacus cucurbitae* under field conditions (1975)

	No. S-males (S)	No. N-males (N)	% egg-hatch		C
			Kudaka Is. (H_c)	Control area (H_n)	
Jan. 1	300	79	24.5	74.0	0.53
14	107	13	7.0	53.8	0.81
29	265	38	11.1	86.7	0.97
Feb. 5	275	36	9.2	56.4	0.67
Mean					0.75 ± 0.16

$$C = \frac{H_n - H_c}{H_c - H_s} \cdot \frac{N}{S} \quad (H_s = 0)$$

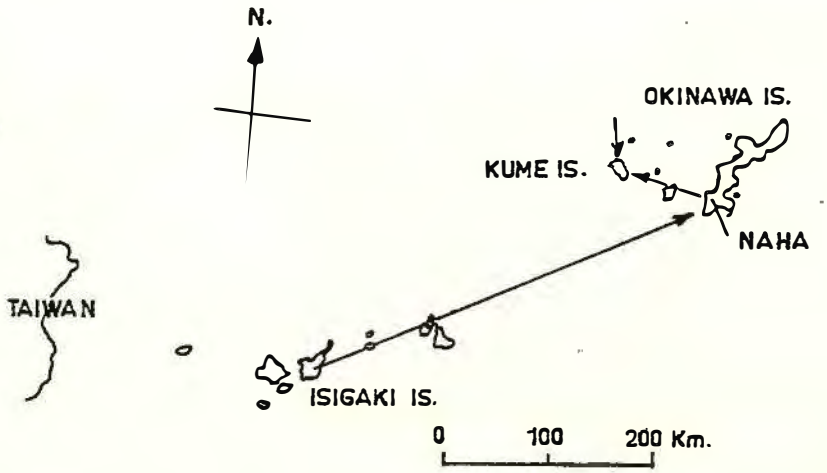

Fig. 1.- Map of Ryukyus

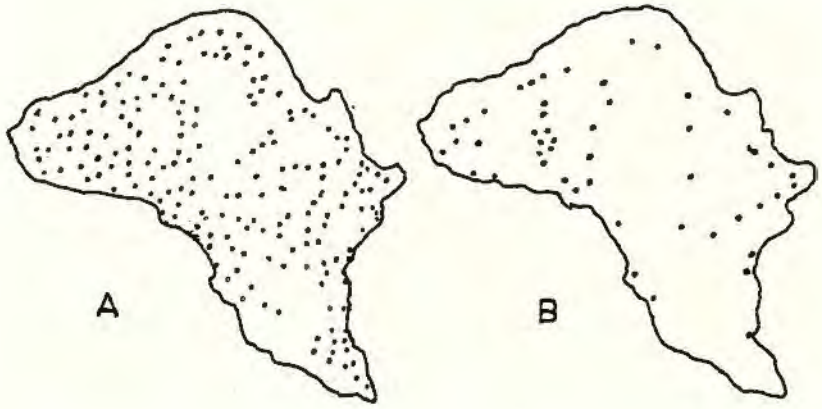

Fig. 2.- A: Points of release of sterile flies on Kume Is. Open circles indicate added release points. B: Localities of monitor traps.

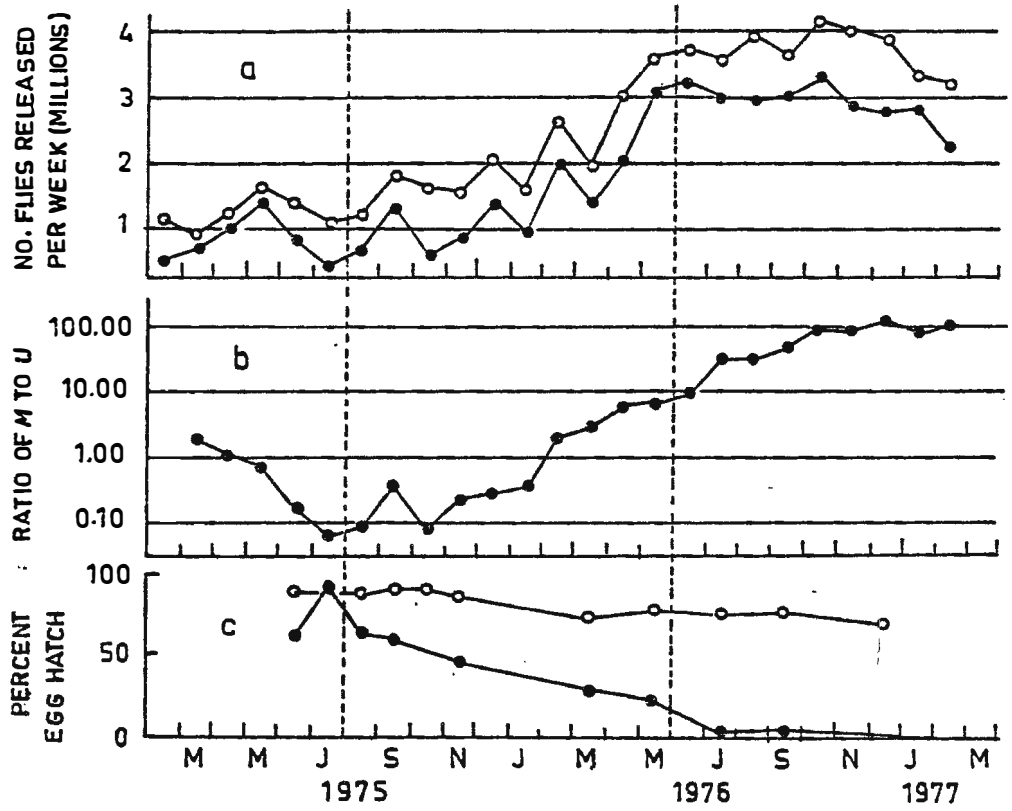

Fig. 3.- Results of sterile insect releases on Kume Is. from February, 1975 to February 1977. Fig. 2-a shows the average number of sterilized pupae released per week (open circles) and estimated number of flies emerged (solid circles). Fig. 2-b shows the monthly ratio of marked (*M*) to unmarked males (*U*) caught in 50 monitor traps on Kume Is. Fig. 2-c shows the hatchability of eggs laid by female melon flies collected on Kume Is. (solid circles) and Naha, the control area (open circles).

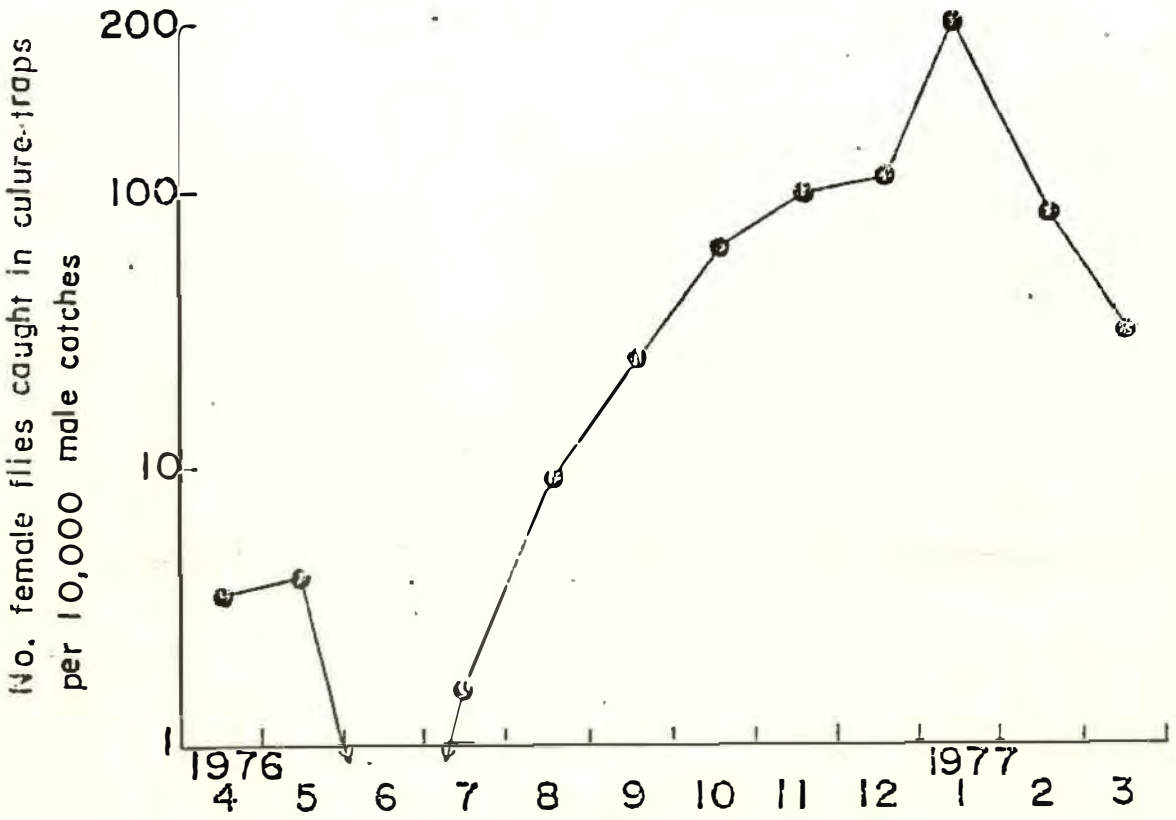

Fig. 5. Female melon fly catches in culure-traps during the SIRM on Kume Is., Okinawa

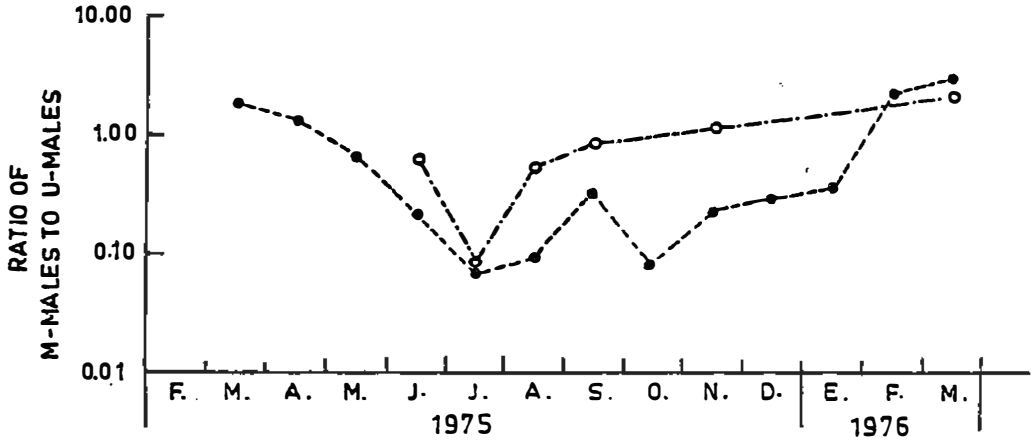

Fig. 4.- Monthly changes in the ratio of marked to unmarked males caugh in monitor traps (solid circle). Open circles indicate the estimated ratio of sterile to normal males and solid triangules indicate the estimated ratio of sterile to normal females.

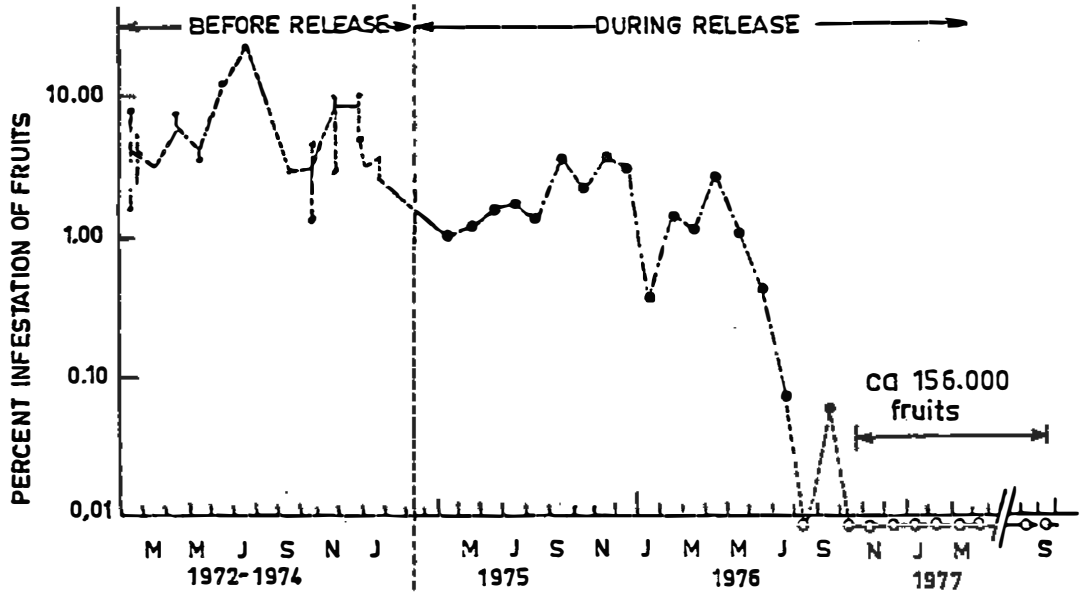

Fig. 6.- Percent infestation of wild cucurbit fruits, *Bryonopsis laciniose*, on Kume Is. before and during the mass releases of sterile insects.

CURRENT STATE OF BIOLOGICAL CONTROL OF OLIVE SCALES

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Abstract

Among the olive scales *Saissetia oleae* (Oliv.) is undoubtedly one of the major pests.

After the biological control project of *Parlatoria oleae* (Colv.) in California, researches and utilizations of natural enemies of olive scales mainly concern *Saissetia oleae*.

The so-called "*Saissetia oleae*" involves a complex of species, all with the dorsal H mark on the body. Since a few years it is known that in the Mediterranean area *S. oleae* s. str. is the predominant black scale. Heavy infestations of this Coccid have been recorded in the last years mainly in Italy, Greece, Israel, Portugal, Spain, France, etc.

In spite of the rich parasite complex of *S. oleae* found in South Africa, where the black scale is not a pest, a rather poor number of parasites has been recorded in other regions.

After the long history of *S. oleae* biological control in California, recent attempts have been made in some Mediterranean countries, mainly in Israel, Greece, France, Italy. In the last years some parasites have been imported and released (*Diversinervus elegans* Silv.; *Metaphycus helvolus* Comp., *M. bartletti* Ann. and Myn;, *M. lounsburyi* How., *M.*aff . *stanleyi* Comp., *Coccophagus pulvinariae* How., etc.).

The mass rearing methods of these parasites have been improved.

It is too early to evaluate the results achieved with the recent introductions of natural enemies for the biological control of *S. oleae* in the Mediterranean regions. Nevertheless, at least in some areas, the programme should be intensified in the framework of an international cooperation.

LA LUTTE BIOLOGIQUE CONTRE *Prays oleae* ET *Dacus oleae*

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Les problèmes des déséquilibres biologiques posés par l'utilisation abusive des insecticides ont conduit, depuis une vingtaine d'années à envisager l'utilisation d'agents biologique contre *Prays oleae* et *Dacus oleae*.

Lutte biologique contre *P. oleae*

Les recherches ont porté sur l'étude des possibilités d'utilisation d'un Braconide, *Chelonus eleaphilus*, dont la production a pu être assurée sur hôte de laboratoire et sur la mise au point de l'élevage d'un Chalcidien, *Ageniaspis fuscicollis praysincola*.

Par ailleurs, sont également étudiées les modalités d'utilisation de parasites oophages (Trichogrammes), les travaux actuels étant surtout consacrés au choix d'un écotype adapté, bien que des lâchers expérimentaux aient déjà été réalisés.

Lutte biologique contre *D. oleae*

Elle a été marquée par les travaux et les expérimentations des Stations d'Antibes et de Palerme entre les années 1960 et 1968, qui ont eu pour point de départ la mise en élevage d'*Opius concolor*. Des élevages de ce Braconide ont été alors installés progressivement dans l'ensemble du bassin méditerranéen : France, Italie, Grèce, Espagne, Liban, Yougoslavie.

L'action d'*O. concolor* paraît généralement variable, même dans les zones où il n'est pas en compétition avec les Chalcidiens ectophages, mais il paraît toutefois difficile de vouloir tirer des conclusions définitives en raison des conditions expérimentales insuffisantes et des contrôles souvent trop superficiels.

Cependant, on peut estimer, semble-t-il, qu'*O. concolor* a une efficacité suffisante pour maintenir les populations larvaires à un niveau assez bas et que dans un concept de lutte intégrée il pourrait permettre de retarder les applications chimiques à la période d'explosion automnale de *D. oleae*.

Il serait souhaitable d'orienter la lutte biologique contre *P. oleae* et *D. oleae* vers la recherche de parasites (espèces, sous espèces ou écotypes) déjà adaptés à la zone de répartition de l'olivier et de réviser les techniques d'utilisation de ces parasites.

THE MEDITERRANEAN FRUIT FLY *Ceratitis capitata* WIED. IN EGYPT

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Summary

The Mediterranean fruit fly *Ceratitis capitata* Wied. is considered nowadays as one of the important pests attacking fruit crops in Egypt. Recent studies were directed towards the modern approaches for its control such as the application of the sterile male technique. The present paper reviews the history of *C. capitata* in Egypt since its introduction in 1904. It also embodies the results of recent studies relevant to the application of genetic methods in general and the sterile male technique in particular.

Ecological studies included the seasonal abundance in relation to host plants, attractant lures and climatic factors together with adult dispersion and flight range. Mass culturing techniques results were discussed from different points of view. Biological studies included the effects of temperature on immature stages together with adult longevity, fecondity and behaviour. The biological effects of gamma irradiation were discussed aiming at establishing a sterilizing dose of the considered strain of Medfly together with the possible histopathological effects of radiation on both female and male reproductive organs.

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FRUIT FLIES CONTROL BY CHEMICALS ATTRACTANTS AND REPELLENTS*

Ugo CIRIO and Giovanni VITA

Introduction

"Attractants and Repellents" is not a recent chapter in applied entomology. Experiments to control fruit flies with olfactory lures or chemical repellents substances have been pursued since the beginning of the century (BERLESE, 1905, 1907 ; BOHORQUES, 1934 ; RICHARDSON, 1916 ; FROST, 1929 ; COSTANTINO, 1930 ; BUA, 1932 ; McPHAIL, 1939 ; BOYCE, 1934).

However there is a great concern today in the world about this kind of studies and applications. Involved in this concern are the insecticides pest control, the approach of multidisciplinary research of the agroecosystems, the pursuit of more selective means of pest management aiming at improving a better environment quality.

Long study and experience showed that fruit flies attractants and repellents have to be considered in the light of research based on the insect/plant relationships (KOGAN, 1977) or/and in the area of chemical ecology (DUFFEY, 1977). Therefore these discussions are included in any broad treatment covered by the role of chemical factors in insect/plant relations (BECK et al., 1976 ; DETHIER, 1970 ; CHAPMANT, 1974 ; GILBERT et al., 1975 ; PROKOPY, 1977 ; SHOONHOVEN, 1972 ; SOUTHWOOD, 1973 ; VAN EMDEM, 1973) or in more specific review (BEROZA, 1972 ; HENDRY, 1976 ; METCALF et al., 1975).

The aim of this talk is to limit the review of attractants and repellents to fruit flies damaging crops in Europe and in the countries of Mediterranean basin, namely the Trypetidae *Ceratitis capitata* Wied., *Dacus oleae* Gmel, and *Rhagoletis cerasi* L.

First we should consider the status of research and application of these compounds, then their chemical identity and behavioural function, and finally we will discuss their use in ecological terms.

Status of laboratory and field research

Attractants and repellents both operate via behavioural responses based on the great specialization of chemioreceptor cells of these insects. Considering the aspect of utilization of these products, four control techniques are examined, namely food lures, sex pheromones, oviposition deterrents, and repellents.

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The present status of research related to each species is summarized in Table 1.

a) Food lures. At present several ammonia releasing substances as protein and yeast hydrolysed, and ammonium salts, are largely used either to control all these species (bait spray) or to monitor adults (trapping). Therefore it is unclear the real power of attractiveness of these products, their persistence in the field condition, and their effective range of attraction.

Synthetic species specific lure, that mainly attract only males, is available only for *Ceratitis capitata* (e.g. Trimellure). However, the results of trials carried out to eliminate Medfly with intensive trapping method based on this synthetic lure are contradictory (STEINER et al., 1962 ; ARROYO V., 1970; CIRIO et al., 1972).

Food lures may be useful combined with color stimuli to trap all these flies (PROKOPY, 1977) or added with trimedlure for controlling *Ceratitis capitata* (COHEN et al., 1967).

Recent studies aimed at identifying olfactory attractants from plant extracts involved the *Dacus oleae* (FIESTA ROS de URSINOS et al., 1972 ; STRAVRAKIS, 1976 ; VITA et al., 1976) and *Ceratitis capitata* (KEISER et al., 1975). Among this kind of products may be also included the aglucone, an olive substance obtained from the hydrolysis of oleoeuropeine, found active towards the females of *D. oleae* (GIROLAMI et al., 1975) but probably attractive also towards the male. Recently bioassay tests with synthetized substance of *Musca domestica* sex pheromone have shown significant attraction towards the adults of *Dacus oleae* (NICCOLI, 1975).

b) Sex pheromones. Although in pest control the fruit flies sex pheromones are considered less powerful than lepidoptera sex pheromones, the study on this area is progressing very well.

In *Ceratitis capitata* the various components of pheromone mixture have been individuated (JACOBSON et al., 1973). However, while in the laboratory these compounds attracted the virgin females, in field tests they attracted more males than females (OHINATA et al.,1977). In this species it has also been found that some terpenes act as male excitants (GUIOTTO et al., 1971 ; VITA, in press).

The research of *Dacus oleae* sex pheromone is encouraged by lack of a species specific attractant available for this fly. In Greece the investigations on the gland source of this odor (ECONOMOPOULOS et al., 1971) were followed by the isolation of pheromone blend (HANIOTAKIS, 1974) and of bioassay tests either carried out in the laboratory or in the field condition (HANIOTAKIS et al., 1977 ; HANIOTAKIS, 1977).. In Italy the National Council of Research has set up a multidisciplinary programme to identify the *Dacus* sex pheromone (CNR, 1977).

So far, several chemical compounds have been identified (VITA et al., in this meeting), some of them synthetized (ROSSI et al., in press), and behavioural bioassayed in the laboratory (VITA et al., in this meeting). These researches progressed rapidly when the pheromone material was directly collected from gland source instead than from the odor of the caged flies (VITA et al., in this meeting). In this species there is little evidence for the function of male sex pheromone (HANIOTAKIS, 1977).

In *Rhagoletis cerasi* the presence of male sex pheromone has been achieved (KATSOYANNOS, 1976).

Therefore the use of these pheromones to control fruit flies seems to have limitations in the effective range of communication and in the numerous chemical substances involved in the pheromone blend.

c) Oviposition deterrents. This approach to fruit flies control is developing on the behavioural studies of Trypetidae reproduction function. In these insects the egg dispersion among available fruit-hosts is mediated by a marking oviposition deterrent which has the function to regulate the larval intraspecific competition (Table 2).

In *Dacus oleae* after the elucidation that olive juice, spread via proboscis by the female on the olive fruit, is the oviposition deterrent (CIRIO, 1971), some researches followed aiming at founding the active chemical substances of the olive juice (FEDELI et al., 1976, FEDELI et al., 1974 ; VITA et al., 1977). The identification of this oviposition deterrent was achieved by VITA (1978).

The effectiveness of this natural chemical compound has also been confirmed in preliminary field trials (FIUME et al., 1977).

In *Rhagoletis cerasi* like in other *Rhagoletis* species (PROKOPY et al., 1976) the egg dispersion is instead regulated by a marking pheromone deposited by the female following oviposition (KATSOYANNOS, 1975). A partial purified product of this pheromone (HURTER et al., 1976) has been successfully used in cherry trees protection experiment (KATSOYANNOS et al., 1976). The presence of a marking pheromone was also revealed in *Ceratitis capitata* (PROKOPY et al., 1977b).

d) Repellents. The studies on chemical repellents for fruit flies control are very poor. Recently, some natural chemical substances derived from chemical fraction of soy lecitine and waste oil water have been behavioural tested in laboratory (CIRIO et al., 1976 ; 1978), and the last one has been also tested in the field to control the *Dacus oleae* (FIUME et al., 1977). This species was also repelled by a non-host-fruit odor as essential oil (ORPHANIDIS et al., 1970)

In cherry cultivation it has been explored the possibility to repell the *Rhagoletis cerasi* by hanging up under the trees

sponge material imbued with the highest volatile product available (VITA, unpublished data).

At conclusion of this review we have to note that the advances towards this new approach of pest control is slow, may be because the problems associated limit the understanding of complex insect/plant relationships ; besides we must consider the difficulties of finding and synthetizing active behavioural compounds and a certain pessimism to use these tactics in pest management.

Chemical identity and behavioural function

The study of chemical substances affecting insect behaviour is mainly directed towards the establishment of reliable testing procedures.

The approach to relate the field performance of these substances to their chemical characteristics seems to be insufficient. Therefore the quality of chemical signal may be also influenced by the physical and physiological conditions of emitter and receiver organism.

The various insect or plant compounds which act on the behaviour of fruit flies here considered are summarized in Table 3.

Regarding olfactory lures it should be noted that several authors found the odor of a given plant attracts fruit flies. However few are the plant compounds exactly identified as attractants. In addition, the knowledge that decomposition products of food lures are recognized by fruit flies as suitable food (e.g. honeydew, bird excrements) should stimulate research on the mechanism of olfactory releasing stimuli.

The common food lures are characterized by a high chemical instability with final production of ammonia. However since it has been found that the attractiveness of these substances increase when the ammonia gas is emitted continuously and at low concentration (WIETING et al., 1939), an attempt should be done for detecting unexpenses ammonia releasing material that respond to these criteria.

The trypetidae sexual pheromone is characterized by a blend of numerous chemical compounds with different behavioural and chemical functions. In *Ceratitis capitata*, while two volatile compounds, the (E)-6-Nonen-I-ol and the Methyl (E)-6-Nonenonate, emitted by the male have an attractive function, numerous other non volatile substances (fatty acids) exert a chemical influence on the efficacy of the pheromonal blend. This type of pheromone mixture occurs also in other fruit flies sex pheromones.

This pheromone chemical complexity may be a factor in modifying its biological activity in the field experiment.

From an evolutionary point of view interesting considerations are possible to advance about the presence of some compounds (e.g. the "6-Nonen-I-o1) in the sex pheromone of *Ceratitis capitata, Dacus oleae*, and probably *Anastrepha suspensa* (Table 4).

Another case of chemical ubiquity regards the presence of the terpene p-Cymene either in many plant genera or in the pheromone of the olive fruit fly. This knowledge involves that a same chemical background occurs in insect belonging to the same family and among insect and plant living in a common environment.

Regarding the chemical repellents here considered, they may be distinguished according to PAINTER (1967) as : a) volatile substances that repel the insect without coming in contact with it (olfactory repellent), b) non volatile substances that repel the insect only after direct contact with it (chemotactic repellent).

All the deterring oviposition substances (pheromones and kairomones) are included in the group of chemotactic repellent. At present, among these compounds only the *Dacus oleae* oviposition repellent has been individuated (VITA, 1978). This substance,a phenolic compound with a high degree of efficacity, is generated by enzymatic oxidation of the olive fruit glucoside.

The β-3-4 dihydroxyphenilethyl alcohol as many other phenolics substances act on nervous cells interferring with their metabolism. It has been observed that at low concentration this substance acts as excitorepellent on insect tarsi while at high concentration it functions as an anesthetic. The field use of this kind of repellent is limited by its instability under light.

The low persistence of other fruit flies oviposition deterrent suggests the hypothesis that similar chemical substances may be involved in these products.

Some ecological considerations

The use of chemical attractants and repellents against fruit flies needs a greater background knowledge of insect ecology than application of other control techniques (the sterile insect technique excluded). Particularly we have to consider either the features of population behaviour of such species or the characteristics of crop.

All these species may be defined "r-strategist" pests (BATEMAN, 1976 ; SOUTHWOOD, 1977) according to their short generation time, high reproductive rate, mobility, low mortality caused by biotic factors, and their unstable environment.

Besides the extremely low economic threshold favours the control strategy based on their rapid elimination from the crop. This suggests that attractants methods, like the use of insecticides, are much more effective than other control techniques. In this framework the use of repellents, which pursuits the aim of increasing the "resistence" of plant instead of insect elimination, has always be done in conjunction with the attractants.

However in both cases, the features of crop have to be carefully considered in the light of the great influence of plant-hosts on the behavioural performance of insects. So, the use of repellent shall be avoided when the density of pest population is high or/and when high susceptibility tree varieties are involved (increase of insect adaptability). Viceversa both these situations are favourable to the use of attractants.

Long periode of fruit-host availability to flies is also a disadvantage for the employment of repellent products. This is the case of olive fruit/*Dacus oleae* and Citrus fruit/*Ceratitis capitata* relations. In this regard the cherry crop seems to offer some advantages for using repellents compounds in combination with attractants substances.

In Table 5 are summarized the actions of different kinds of control techniques, as their specificity, efficacity and operational cost, related to he use of these chemical products.

Therefore it is clear that all these new tactics have to be considered in the framework of strategy that lead the crop management.

Summary

The paper review the status of research on the use of attractants and repellents to control the *Ceratitis capitata, Dacus oleae* and *Rhagolethis cerasi*.

The chemical identity of these substances is discussed in relation to their behavioural activity.

Their employment is critized in ecological terms and related to the characteristics of crop and population behaviour. The efficacity, specificity and operational cost of each control techniques are compared.

Tab 1. - Status of Research in the use of chemical substances affecting the behaviour of some *Trypetidae* (x = initiated ; xx = advanced ; xxx = achieved)

Species	Behavioural aspect	Status of research releated to :		
		Chemical identificat.	Laboratory (behaviour,bioassay)	Field evaluation
Ceratitis capitata Wied.	Attractants (Plant odors)	x	xxx	
	Male attractant (Trimedlure)	xxx	xxx	xxx
	Male sex pheromone	xxx	xxx	xx
	Male sex excitant	xxx	xxx	
	Oviposition attractant	x	xxx	
	Oviposition deterrent	x	xxx	
	Repellent	xx	xxx	
Dacus oleae Gmel.	Attractants (Fruit odors)	x	xx	x
	Female sex pheromone	xx	xxx	x
	Oviposition stimulant	xxx	xxx	x
	Oviposition deterrent	xxx	xxx	xx
	Repellent	xx	xxx	x
Rhagoletis cerasi L.	Sex pheromone	xx	xx	
	Oviposition deterrent	xx	xxx	x
	Repellent	xx	xxx	xx

Tab. 2.- Relation between persistence of oviposition deterrent and egg development in some *Trypetidae* species

Species	Persistence of deterrent (days)	Development of egg (days)	Reference on the oviposition deterrent
Anastrepha suspensa	6	3-4	PROKOPY et al., 1977a
Ceratitis capitata	6	3	PROKOPY et al., 1977b
Dacus oleae	5	3	CIRIO (in prep.)
Rhagoletis cerasi	12	7	KATSOYANNOS, 1975
Rhagoletis completa	5	3-4	CIRIO, 1972
Rhagoletis fausta	9	?	PROKOPY, 1975
Rhagoletis pomonella	4	4	PROKOPY, 1972

Tab. 3. - Behavioural aspects and chemical identity of various substances (pheromones and allomones) in some *Trypetidae* species

Species	Behavioural aspect	Chemical
Ceratitis capitata	Male sex pheromone	°(E)-6-Nonen-1-ol,Methyl(E)-6-Noneate,several fatty acids
	Male sex excitant	°Essential oil(Citrus genera,*Arcangelica officinalis*), p-Cymene
	Food lure	°Trimedlure
	Oviposition deterrent	°Not individuated
	Repellent	°Phenolic compounds (β-3,4-Dihydroxyphenilethyl alcohol, 2-Phenil-ethyl alcohol)
		°Soybean lecitine (acetone soluble fraction)
Rhagoletis cerasi	Male sex pheromone	°Not individuated
	Oviposition deterrent	°Not individuated
	Repellent	°Phenolic compounds (β-3,4-Dihydroxyphenyl ethyl alcohol, 2-Phenyl-ethyl alcohol)
		°Soybean lecitine (acetone soluble fraction)
Dacus oleae	Female sex pheromone	°(E)and/or(Z)-6-Nonen-1-ol,p-Cymene, fatty acid esther
	Food lure	°(ethylic ether soluble fraction) of olive fruit
		°Oleoeuropein aglucone
	Oviposition deterrent	°β-3,4-Dihydroxyphenyl ethyl alcohol
	Repellent	°Soybean lecitine (acetone soluble fraction)
		°2-Phenyl ethyl alcohol

Tab. 4.- Some examples of chemical ubiquity interesting some fruit flies

Chemical	Individuated in plant	Individuated in insect	Effect on fruit flies behaviour
(E)-6-Nonen-1-ol		*Ceratitis capitata* *Anastrepha suspensa* *Dacus oleae* (in the sex pheromones)	Attractant and excitant
p-Cymene	*Citrus* genus *Labiatae* genus	*Dacus oleae* (in the sex pheromone)	Attractant and excitant

Tab. 5. - Comparison of action of different kinds of attractants and repellents to control some fruit flies.

Control techniques	Action considered		
	Efficacity	Specificity	Operational cost
1. ATTRACTANTS :			
1.1. SYNTHETIC ATTRACTANT TRAPS (e.g. Trimedlure)	Independent of population density	High	Increase with increasing population density and size
1.2. SEX PHEROMONE TRAPS	Generally limited to low population density	High	Increase with decreasing population density and size
1.3. BAIT SPRAYS (e.g. *Ceratitis capitata, Dacus oleae*)	Independent of population density	Moderate	Increase with increasing population density and size
2. REPELLENTS :			
2.1. OVIPOSITION DETERRENT (e.g. *Rhagoletis cerasi, Dacus oleae*)	Limited to low population density	High	Increase with increasing population density and size
2.2. UNSPECIFIC REPELLENTS (e.g. Soy lecitine)	Limited to low population density	Low	Increase with increasing population density and size

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Dacus oleae SEX PHEROMONE : ISOLATION AND PARTIAL CHEMICAL IDENTIFICATION

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The chemical identification of the compounds contained in the pheromonal blend, emitted by the olive fly female, is the goal of a research group organized by CNR (Italian National Research Council).

The females sex pheromone was collected using two different methods :

a) recovering volatile insect pheromone from large females population (± 3.000 females) with a liquid nitrogen, cold trap ;

b) extracting pheromone from the anal gland (associated with the rectum) of 6-10 days old females.

Both pheromonal blends obtained from the Et_2O soluble fraction of the females condensate and female glands are analyzed by gas chromatography, utilizing a Dani 3600 model equipped with a flame ionizzation detector and a 45 x 0.3 mm capillary glass column operated with H_2 carrier gas (flow rate of 30 ml/min). Among the numerous compounds identified two were tested for their biological activity : the 6-Nonen-1-ol, and the aromatic compound p-Cymene. The first was detected at lower concentration in the sample collected with the cold trapping method and characterized with a fragmentographic analyses using a LKB 2091 B mass-spectrometer associated with a computer "Digital 2130 model". The Stereoisomerical form (E) or (Z) was not determined. The second was individuated in large amount both in the condensate odor of insects than in the pheromonal glands. These substances synthetized and tested in olfactometer for their attractiveness showed high degree of efficacy. Changement in the male adult behaviour was registered when one of the compounds identified was present in the environment : increased locomotor activity, drawing the wings, increased aggressiveness, repeated attempts of copulation, also with other males. The attractiveness of compounds identified toward the adult olive flies (males and females) was tested in a 216 cm^3 plexiglass cubic olfactometer (Beroza and Baker model). The bioassay test was carried out by registering the visits exploited by the flies on paper roll with a closed circuit television system in the last three hours of the diurnal photophase.

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Table 1 - Attractiveness to adult olive flies of different substances tested in olfactometer

Treatment	Chemical formula	Visits number (ii) (male)
Pheromonal glands (i) (iii)	-	11
p-Cymene (iii)	$C_{10}H_{14}$	8
6-Nonen-1-ol	$C_9H_{18}O$	10
Blank	-	2

(i) Five glands crushed on paper roll
(ii) Total of three hours of observation with singular population (100 males + 100 males)
(iii) One nanoliter of the compound diluited in n-pentane.

Table 1 shows that all tested compounds were attractive toward olive flies. The presence of 6-Nonen-1-ol in the sexual pheromone of other *Trypetidae* as *Ceratitis capitata* (Wied) and probably *Anastraepha suspensa* (Loew) lead the hypothesis that like in the family of other insect (*Lepidoptera, Tortricidae*), some specific chemical substances occurrant in the pheromones of several *Trypetidae* species. Field evaluation of these new attractants will be carried out.

THE PHAGO-STIMULATORY EFFECTS OF PROTEIN HYDROLYSATES AND THEIR ROLE IN THE CONTROL OF MEDFLIES

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Many species of flies, including tephritids require proteins for normal development of their eggs. DETHIER (1976) in his recent book "The Hungry Fly" summarizes the limited amount of work which was aimed at elucidating the physiological phenomena involved in this process.

The present paper deals much less with the physiological phenomena and concentrates mainly on behavioral responses of *Ceratitis capitata* to proteins, since these are most relevant to the control of the fly.

Protein hydrolysate-malathion bait sprays are used in many parts of the world as the standard technique for the control of infestation of various fruit flies.

Bait sprays based on mollasses have long been used for the control of fruit flies, but without great success. A breakthrough come with the discovery of McPHAIL (1939), who found that the attractiveness of impure fermenting sugars was mainly due to the presence of proteins in the mixture, and that tephritids are attracted to decomposing proteins. In the fifties acid-hydrolyzed proteins from corn syrup were developed by STEINER (1955) and by GOW (1954) and a commercial product manufactured by STALEY (PIB-7) based on their development has become the standard baiting and trapping lure for various fruit flies.

According to GOW (1954) the attraction of proteinaceous materials appears to be chiefly due to products of microbiological action. This is in agreement with the observations of STEINER (1952) that the material loses its attractiveness as it loses its tackiness. Yet, bait spray deposits after drying continue to attract and kill flies for 1-3 weeks, depending upon rainfall (STEINER, 1955).

It seems that protein hydrolysate may work as attractants as well as arrestants. As long as it is wet and emits volatile materials it exerts attraction over a considerable distance (STEINER, 1955) ; the dry deposit most likely works as an arrestant due to the phagostimulatory effect of some of its components. Flies coming in contact with the treated area will stay there long enough to ingest or absorb a lethal dose of the malathion.

While Bateman's team in Australia is trying to determine the chemicals responsible for the attractancy to fruit flies to the volatiles emitted from protein hydrolysate, our study deals with the chemicals which are responsible for the phagostimulatory effect of protein hydrolysates.

Strong phagostimulatory effects of protein hydrolysate and yeast hydrolysate were observed with the house fly (*Musca domestica*) (ROBBINS et al., 1965). Filter paper discs impregnated with yeast or protein hydrolysate did not show any attraction to the flies from distance, however when placed in a cage of 4-7 days old sugar fed flies, flies that came in contact with the disc fed vigorously, and within a few minutes large cluster of flies accumulated on the disc. 98 % of the clustered flies were females. ROBBINS et al. (1965) identified guanosine monophosphate as the major active component of yeast hydrolysate, and several amino acids, including leucine, methionine, lysine and isoleucine, as the active components of casein hydrolysate.

In a similar experiment with yeast or casein hydrolysate with 4 day old Mediterranean fruit fly, no aggregation of the flies was observed. This agrees with the early observations of STEINER (1955) that bait sprays applied to artificial surfaces more than a few inches from the nearest leaves are ineffective. In order to evaluate the phagostimulatory effect of protein hydrolysates we developed the following assay ; Protein hydrolysate or any other candidate material is dissolved, in the required concentration, in hot water containing 3 % agar, and is poured into petri dishes, 3.5 cm in diameter. Control dishes contain agar alone. One treated dish and one control are introduced into a net cage (30 x 30 x 30 cm) containing 300 flies. The flies aggregate slowly and feed on the protein hydrolysate. 5 counts at a one hour interval are taken, and the cumulative count is considered as the aggregation index. Illumination should be strong and temperature should be around 28°C in order to get reproducible results. At the end of the 5 hours the petri dishes are removed, and sugar and water are given to the flies. If the same group of flies is used again on the following day, the dead flies are removed and replaced by flies from the same stock which is maintained on sugar and water.

As a standard we used Sigma enzymatic casein hydrolysate. As a first step, the optimal concentration of this hydrolysate was determined. Within the concentration range studied, using mixed populations of males and females, we found that 2.5 % casein hydrolysate was the optimal concentration, giving an aggregation index of over 100for flies aged 4-6 days. It is interesting tnat concentration of 5 % was less attractive, perhaps due to too high a concentration os some non-palatable amino-acids (Table 1).

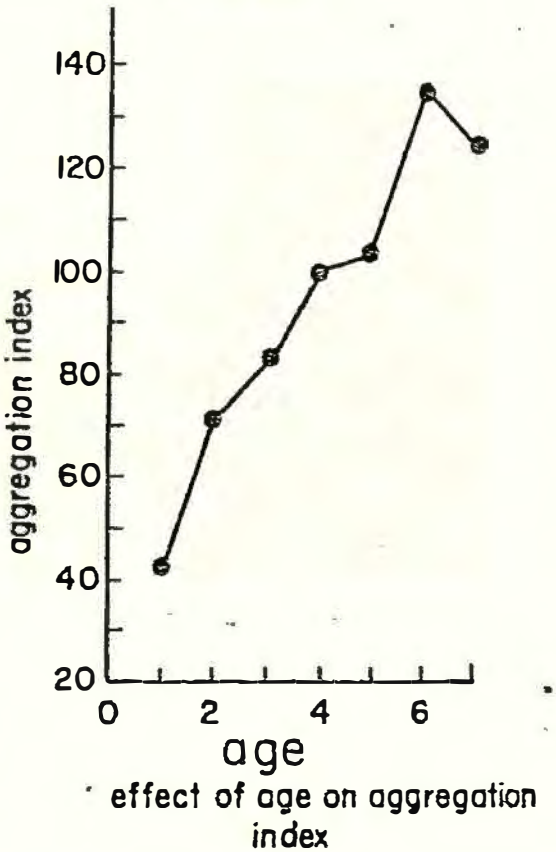
Table 1. - Effect of concentration of casein hydrolysate on aggregation of the flies

age (days)	concentration of casein hydrolysate			
	1.5 %	2.5 %	3.5 %	5 %
3	-	105	-	82
4	82	120	87	21
5	93	103	80	45
6	60	135	103	-
7	118	122	-	-

In view of this finding we used 2.5 % casein hydrolysate as a standard for all the later experiments. The same technique was used to study the effect of age and sex on the response of the flies to protein hydrolysates.

As seen from Fig. n° 1, the aggregation response of the flies to casein hydrolysate increases in the first 4-5 days, and remains high afterwards.

Fig. 1



effect of age on aggregation index

Aggregation of houseflies was studied only at the age when they reached sexual maturity, and we can't tell how the young flies behave concerning aggregation.

If aggregation reflects the quantity of protein ingestion, it is expected (DETHIER, 1961 ; YAMAMOTO & JENSEN, 1967 ; ROBERTS & KITCHING, 1974), to be related to the development cycle of the ovaries -i.e. to increase for the first few days. Since in our procedure the flies are fed very little proteins (the hydrolysate in the agar is not so readily available) - then once reaching the 4th day, the population is maintained in a steady state - and thus retains the high response to protein hydrolysate.

If instead of maintaining the flies on sugar alone they were given a diet containing a mixture of yeast-hydrolysate and sucrose, their aggregation response to casein hydrolysate was drastically changed (Table 2). Feeding for a few hours only on yeast-hydrolysate is enough to abolish the attraction to casein hydrolysate, and when given a choice between sugar and casein hydrolysate, they show a distinct preference for the sugar.

Table 2.- Effect of the diet on responses of medfly (no. represent aggregation index)

age (days)	Flies fed sugar				flies fed yeast hydrolysate & sugar			
	casein hyd.	agar	casein hyd.	sugar	casein hyd.	agar	casein hyd.	sugar
1	67	0	51	49	7	0	12	41
2	117	9	66	35	18	0	15	61
3	85	12	74	43	11	1	11	76

This quick change in response of medflies may perhaps be related to the fact that this fly, unlike most other tephritids, is able to produce and deposit some eggs even when fed on sucrose alone. Metabolite reserves are evidently transferred from larval feeding through the pupae to the adults via the fat body (LANGLEY et al., 1972). Thus, it is possible that already a small supplementation of proteins bring the young fly to a physiological state equivalent to that shown by other flies with developing eggs following protein feeding - i.e. predominantly carbohydrate motivated. This change in response can be explained also by the deficit hypothesis, suggested by DETHIER (1976). DETHIER assumes that flies of each sex emerge from the pupal stage with an accumulated protein deficit. In response to this deficit both sexes are sensitized to protein. In a newly emerged female the deficit is presumed to be larger because of a start of ovarian development. As protein is ingested in response to this initial deficit - the corpus allatum and median neurosecretory cells are activated and the fat body begins to synthesize special

proteins, thus increasing the deficit. Thus demands increase until eggs are formed and the cycle is broken. However, unlike the blowfly or the housefly, the specific proteins already found in the fat body of the newly emerged fly - break the first cycle, and only the second cycle will show closer similarity to other fly species.

The ecological significance of this observation and its role in the control of the fly has to be studied.

If the responses of the flies to the volatiles also changes, as a result of protein ingestion, than the stage most susceptible to the part-poison treatment would be the sexually immature flies, while the females which are about to oviposit will be the least attracted. It is important to collect flies in protein hydrolysate traps and study their reproductive state.

The effect of sex of the fly on its response to casein hydrolysate was studied. Males were separated from females at the pupal stage and the aggregation responses of each were studied. In parallel, the ingestion of casein hydrolysate and glucose of virgin males and females was compared using the j shaped volumetric pipette technique in the same manner employed by GOTHILF et al. (1971). In both criteria males behaved similarly to virgin females - the aggregation index of both was close to each other (though somewhat lower for males) and considerably lower than that of copulated females or mixed population (Table 3).

If virgin females are denied access to protein until they are 7 days old the preference for protein becomes more marked, for 1-2 days. Aggregation index of 180 was obtained with such females as compared to 75 of virgins offered 2.5 % casein hydrolysate for 5 hours every day.

Table 3. - Effect of sex on aggregation index of *C. capitata* to casein hydrolysate

age (days)	males	virgin females	non-virgin females
1	36	36	-
2	28	46	-
3	42	52	-
4	-	-	-
5	69	86	112
6	58	67	-

Protein is ingested by both sexes of flies from the day of emergence, though the female ingests higher volumes. In the male protein ingestion seems to gradually diminish with age - reaching zero around the 8th day (Fig. 2). Toward this age its

carbohydrate uptake is also decreased, while the female which lives much longer still maintains a steady state of sugar ingestion, as well as protein ingestion. Unfortunately we have not measured the ingestion of nutrients by mated females, though they are expected like the housefly (GREENBERG, 1959) or the glowfly (DETHIER, 1976) to consume much larger volumes of protein solutions, which oscillate with the reproductive cycle.

Protein or yeast hydrolysates are mixtures of many chemicals, including all amino-acids. Bateman has found, by replacing the protein with mixtures of pure amino-acids identical with those found in bovine serum albumin, that methionine is an essential component for attractancy of flies to traps. A mixture of methionine and six other amino-acids has been found to have a significant attractancy, due to the (unidentified) volatiles produced from this mixture.

We tried to identify the amino acids which are responsible for the aggregation due to their phagostimulatory effect ; thus, instead of the casein hydrolysate, the 10 amino-acids found in casein were offered to the flies instead of the hydrolysate. We made a mixture containing equal amounts of each acid, rather than trying to imitate the proportions found in casein, and incorporated either on 2.5 % and 5 % of the mixture in the agar. As seen in Table 4 the mixture produced aggregation, yet it was considerably lower than that of the hydrolysate. Assuming that some of the amino acids may be deterents in the concentration tested. When given a choice between 2.5 % casein hydrolysate and 2.5 % amino acid mixture the flies showed marked preference to the hydrolysate (Table 5).

Table 4. - Aggregation response of *C. capitata* to amino acid mixtures (when paired only with agar as control)

age (days)	aggregation index		
	2.5 % casein hydrolysate	18 amino acids (x) (5 % for the first two days, 2.5 % after)	5 amino acids (xx) (5 % conc.)
1	43	17	12
2	72	50	40
3	83	-	40
4	100	-	50
5	103	62	-
6	135	70	-

(x) alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenyl-alanine, proline, serine, threonine, tryptophane, tyrosine, valine (in L form)

(xx) isoleucine, leucine, lysine, methionine, phenyl-alaline

Fig. 2

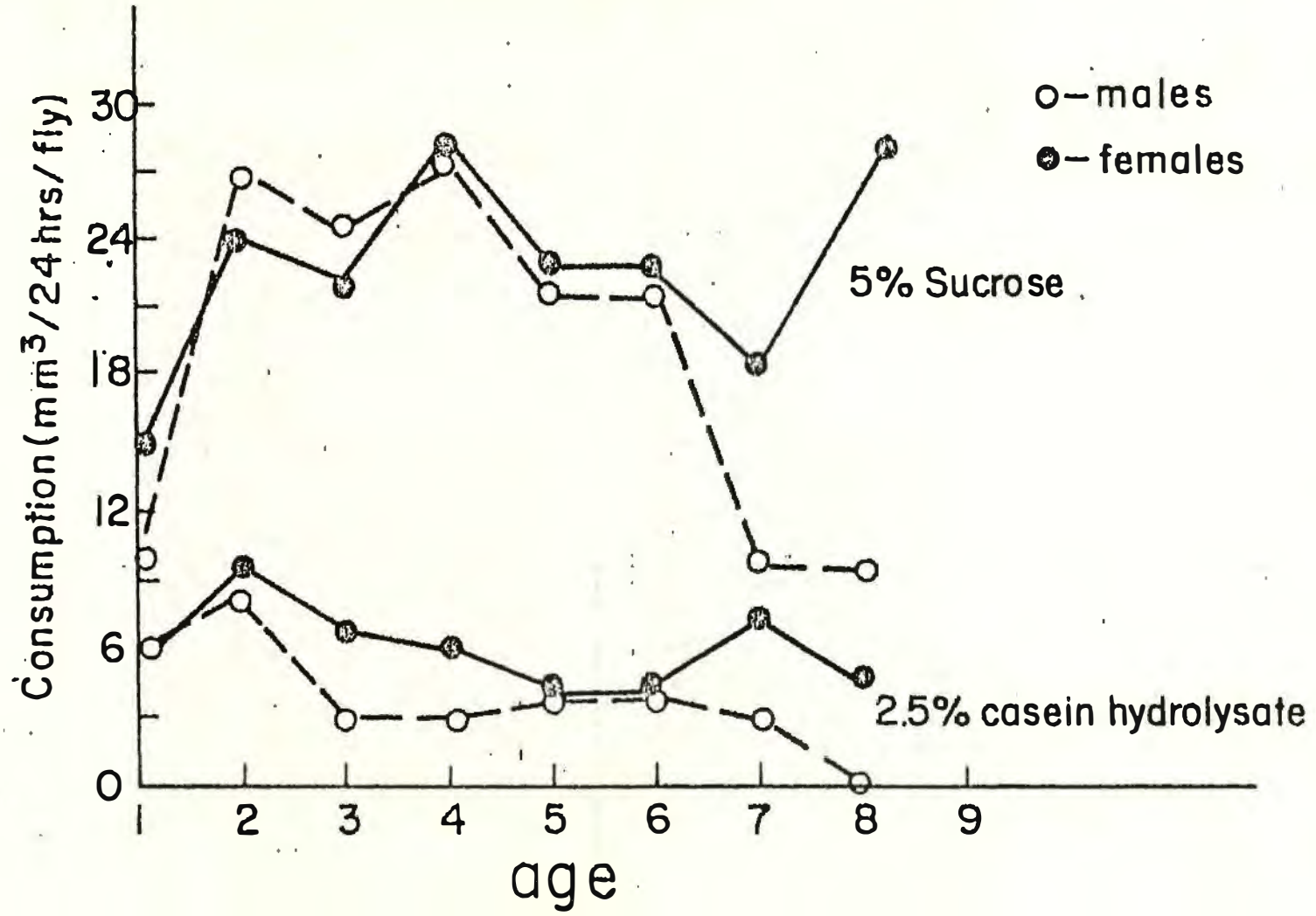

Consumption (mm³/24 hrs/ fly)
30
24
18
12
6
0
1
2
3
4
5
6
7
8
9
age
o—males
●—females
5% Sucrose
2.5% casein hydrolysate

Table 5. - Selection between amino acid mixture & casein hydrolysate

age (days)	aggregation index		
	2.5 % casein hydrolysate	mixture of 18 amino acids	total
5	77	32	110
6	58	25	83

We tried a mixture of the 5 amino-acids that caused aggregation of the house-fly ; i.e. leucine, isoleucine, methionine, lysine & phenyl-alanine. The mixture, in 5 % concentration was stimulatory, but again much less than the hydrolysate (Table 4). In the house-fly each of the stimulatory acids caused very strong aggregation when presented alone in phosphate buffer. However, none of the 5 amino-acids showed considerable phagostimulatory effect on the medfly, in concentration of either 1 % or 0.1 % (Table 6), an addition of Na_2HPO_4 did not seem to potentiate the very weak effect of these acids. It seems that unlike the house-fly - a mixture of several amino acids is needed in order to evoke feeding response in the medfly.

Table 6. - Aggregation index of *C. capitata* on some amino acids

age (days)	leucine		isoleucine		methionine		lysine		phenyl alanine	
	0.1 %	1 %	0.1 %	1 %	0.1 %	1 %	0.1 %	1 %	0.1 %	1 %
1	5	10	0	0	0	8	0	10	0	8
2	-	22	-	0	-	20	-	0	-	22
3	12	22	10	0	7	15	7	0	8	18
4	22	-	20	-	13	-	15	-	14	-

All the other amino acids were tested singly and none except for arginine gave an aggregation index higher than 20, and therefore was considered as non-stimulatory. Arginine (0.1 %) gave an aggregation index of 45 in 3 day old flies (Table 7). This effect could not be increased by increasing the concentration of the acid, and could not be potentiated by phosphate buffer.

Table 7. - Aggregation index of *C. capitata* on L-arginine

age (days)	conc. of arginine		
	0.1 %	0.5 %	1 %
1	5	19	15
2	11	26	25
3	45	26	30

Arginine was further demonstrated to be phagostimulatory in ingestion experiments. When flies were offered two j shaped volumetric pipettes, one containing 0.25 % sucrose and the other containing 0.25 % sucrose and 0.25 % arginine. The volumes ingested from the mixture were twice as big as those from the sucrose alone. This was true for females as well as males.

It is worthwhile mentioning that arginine does not induce any clustering in the housefly. Further studies on the effect of arginine with other amino acids of low qtimulatory effect one needed, in order to find out if a certain combination is responsible for the stimulatory effect of the hydrolysate. This type of information may also help in assessing the phagostimulatory effect of commercial preparations of protein hydrolysate before they are purchased.

As yeast hydrolysate is also a very effective phagostimulant of *Ceratitis*, we examined the phagostimulatory effect of guanosine-monophosphate which as mentioned, was identified as the major phagostimulant in yeast for the house fly. 2, 3, or 5-Guanosine monophosphates were all ineffective, in a range of concentrations either in the presence or the absence of phosphate buffer.

We are still at the beginning of our study, but we believe, that the observations made so far in the laboratory will help us to understand the responses of the fly in nature.

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SECOND FIELD APPLICATION OF THE OVIPOSITION-DETERRING PHEROMONE OF THE EUROPEAN CHERRY FRUIT FLY, *Rhagoletis cerasi* L.

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Abstract

The objectives of the second field application of the oviposition-deterring pheromone of *R. cerasi*, reported here, were to test its effectiveness on entire trees, to test the influence of the pheromone-concentration, and of the number of treatments applied. For these purposes, 8 cherry trees (late variety) of an orchard at the research station of Wädenswil, were sprayed in 1976 with two different concentrations of a partially purified pheromone solution (4 trees per conc.). One week later 2 trees from each group received a second treatment with the same concentration. As control served unsprayed trees of the same orchard and variety. The examination of the infestation rates at harvest (table 1), showed again a high effectiveness of the pheromone (90.1 % for the best treatment) and also that the effectiveness increased with increasing pheromone-concentration and the number of treatments. The results obtained (5.3 % infestation in the best treatment vs. 53 % in the control) under an extremely high population density and weather conditions favorable for oviposition are encouraging for the continuation of the research on the isolation, identification and synthesis of the active compound(s) as well as for the development of suitable application methods.

Table 1.- Comparative results of infestation rates of cherries treated with oviposition-deterring pheromone in 1976. Two cherry trees per treatment and concentration, 400 cherries examined per sample (unpublisned data).

concentration of pheromone (1)	number of treatments	infestation at harvest (%) (2)	% Effectiveness (Abbott)
0.2 %	2	5.3	90.1
0.02 %	2	6.8	87.3
0.2 %	1	7.8	85.4
0.02 %	1	15.3	71.2
Control	-	53.0	-

(1) Crude solution showing biological activity at concentration down to 0.001 %

(2) Mean value from salt-water tests for extracting larvae and % pupae.

AN INDICATION OF AN OVIPOSITION PHEROMON OF *Rhagoletis cerasi* L. (1)

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Bavarian Institute for soil and plant cultivation, Munich (RFA)

In keeping *R. cerasi* by single pairs it was noticed that 30-70 % of the females do not oviposit into the artificial fruits consisting of thin-walled, black small wax domes. It must be concluded that this fruit dummy does not offer all stimuli of the entire chain of stimuli necessary to cause a female to oviposit in the field. Therefore, it was looked for a chemical compound eliciting oviposition.

An airstream cleaned by a column of active coal was led over unripe cherries and afterwards through a column of "Porapak" as strong adsorbens for possible volatile irritant stuff. Then this Porapak was eluted by a certain amount of acetone or hexan. 2 µl of each of the solutions were put on a wax dome.

Males and females approached the treated and untreated wax domes at the same extension. The hexan-treated domes contained significant fewer eggs than the control domes. However, the oviposition rate at acetone-treated domes was 20 % higher than that of the control. In later experiments the control domes have been treated with the pure solvent. Hereby the treated domes were significantly superior to the untreated ones by about 10 %. An increase of the amount of solvent used to 4 and 8 µl diminished oviposition.

(1) Work was supported by the German Bundesministerium für Forschung und Technologie

Prof. Levinson's help in experimentation is gratefully acknowledged.

THEORETICAL METHOD FOR DEVELOPING VISUAL ATTRACTANT TRAPS FOR INSECTS

Herndon R. AGEE

Joint Working Groups on *Ceratitis capitata, Rhagoletis cerasi*, olive pests, and Genetic Methods for Insect Control, International Organization for Biological Control of Noxious Animals and Plants. Western Palaerctic Regional Section. Held at Sassari, Italy, May 15-20, 1978.

Since early history, man has been trying to prevent the insect from reaping the fruits of his labor. The development of methods to destroy insects is contantly progressing from the mechanical crushing of the insect pest under the foot of man, to selective use of pathogens, poisons, parasites, pheromones and visual attractant traps. We will examine the development of visual attractant traps.

Before the invention of the electric lamp--lanterns and lamps with various shapes were used to attract some night flying insects. When the brighter electric lamps became available more nocturnal insects were caught in the visual traps. With the development of the ultraviolet fluorescent lamps, more nocturnal insects were selectively attracted to light traps. As yet, no light trap has been developed that is sufficiently efficient that economic control can be declared. However, light traps are routinely used to monitor populations of some nocturnal insects.

The identification of the most efficient visual trap for day flying insects has been by comparative testing. I will now describe the theory that I use in the development of a visual trap.

First, the eye, the visual receptor, is studied morphologically and histologically.

Second, the electrophysiological techniques that are available today are used to measure the spectral sensitivity of the receptor to a broad spectrum of wavelengths of light from 350 nanometer (nm) to 675 nm.

Third, the spectral reflectance characteristics of host plants and fruits of the insect are measured with a spectral reflectance spectrophotometer to determine what colors the insect selects in nature. (The insect is also receiving other stimuli through non-visual receptors, see attachment (A)).

Fourth, the information obtained on the spectral sensitivity of the insect's eye and reflectance of the host plant or fruit are examined to determine the wavelengths that are reflected by the plant and that are easily detected by the insect eye.

Fifth, a candidate paint is then selected that simulates the spectral characteristics of the host or exceeds the reflection in the wavelength region that is considered to be the receptive region of the insect.

Sixth, laboratory and/or field testing of efficiency of the candidate visual attractant and other attractants that would not be expected to be efficient visual attractants are compared.

Seventh, evaluate the field response in terms of spectral sensitivity, spectral reflection of insect host, and spectral reflection of candidate visual attractants are compared.

Through this method we may be able to derive a principle for routinely determining the most efficient attractant for our pest insects.

Literature on the development of visual attractants will be summarized and reviewed for fruit flies and other insects.

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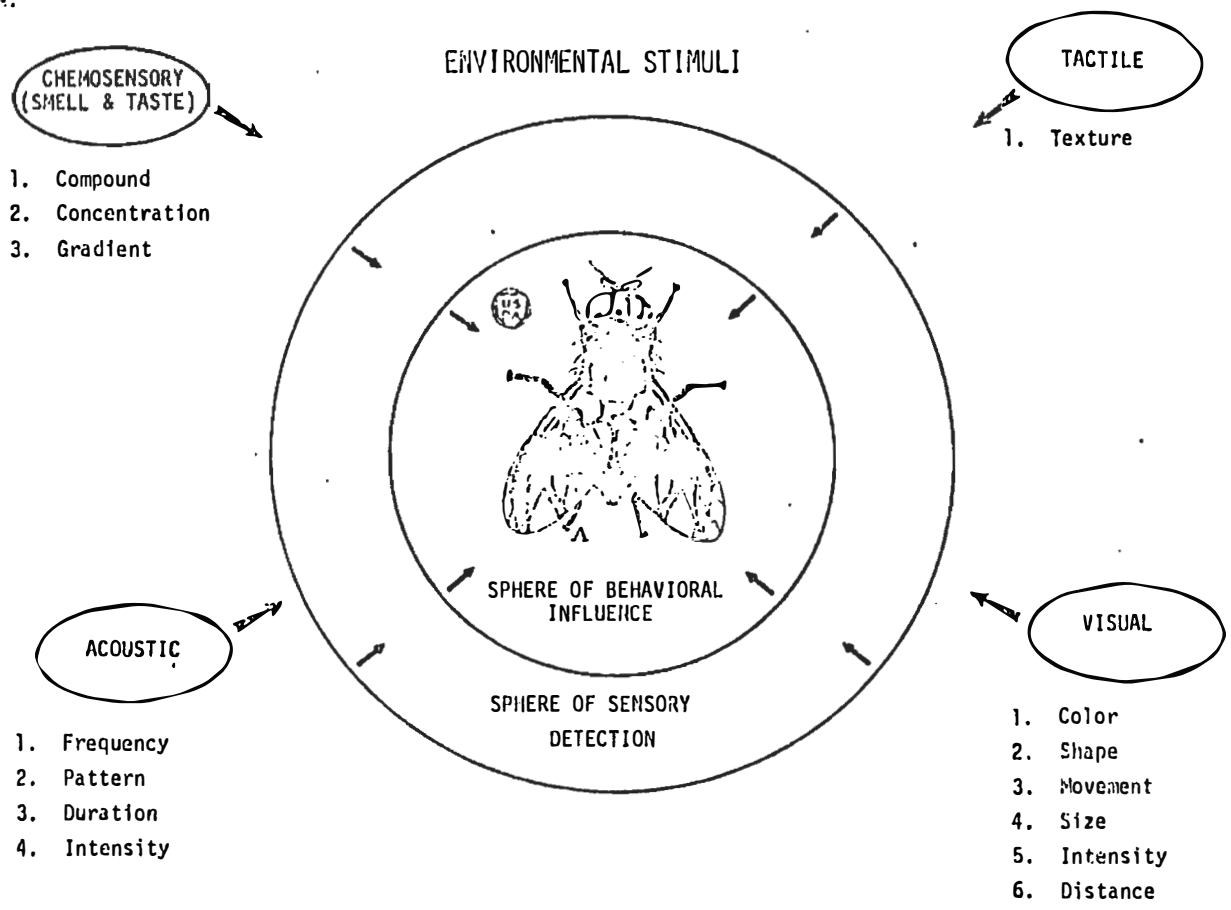
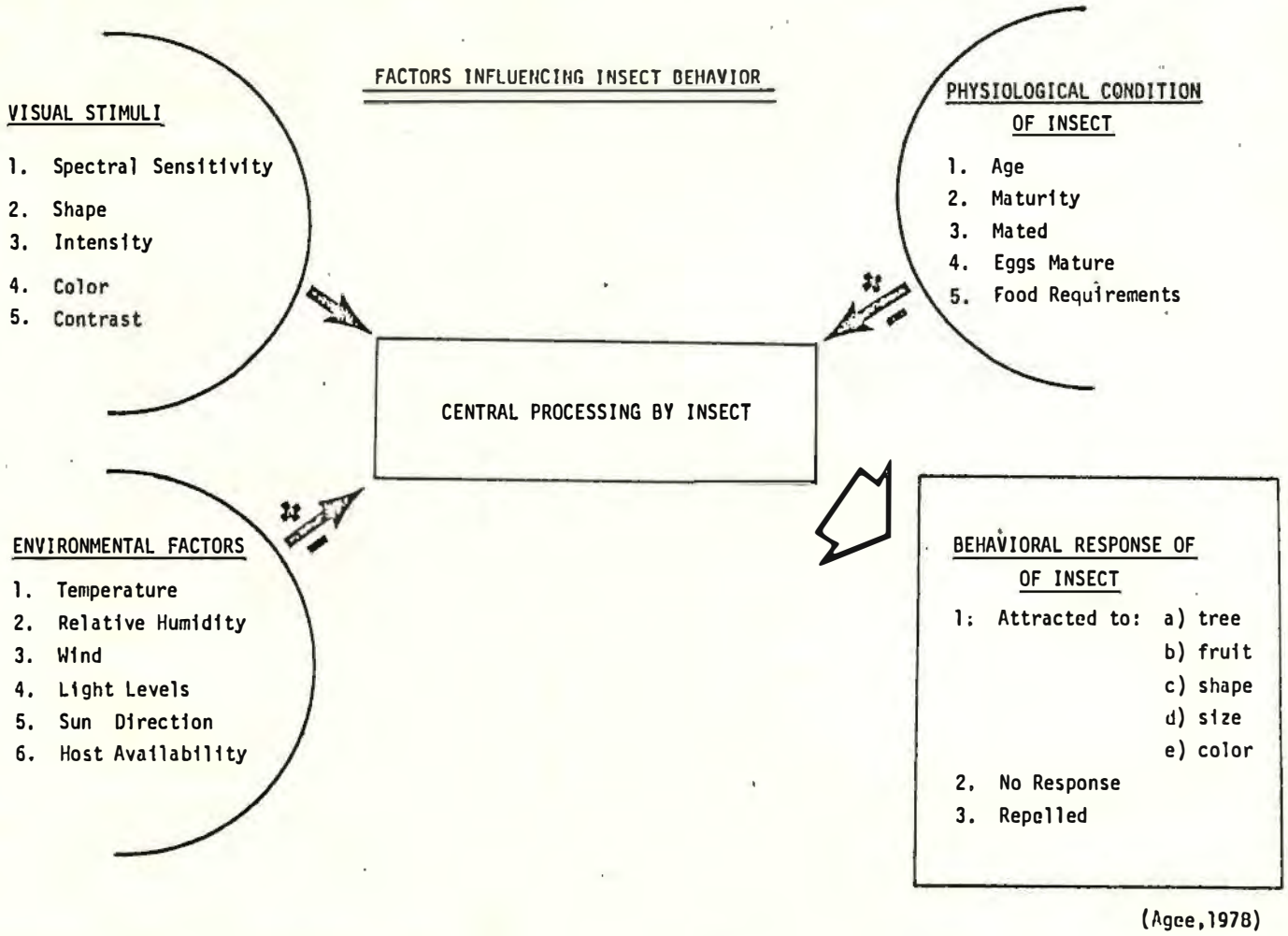
Attachment A.




FACTORS INFLUENCING INSECT BEHAVIOR
VISUAL STIMULI
1. Spectral Sensitivity
2. Shape
3. Intensity
4. Color
5. Contrast
PHYSIOLOGICAL CONDITION OF INSECT
1. Age
2. Maturity
3. Mated
4. Eggs Mature
5. Food Requirements
CENTRAL PROCESSING BY INSECT
ENVIRONMENTAL FACTORS
1. Temperature
2. Relative Humidity
3. Wind
4. Light Levels
5. Sun Direction
6. Host Availability
BEHAVIORAL RESPONSE OF OF INSECT
1: Attracted to: a) tree
b) fruit
c) shape
d) size
e) color
2. No Response
3. Repelled

(Agee, 1978)

VISUAL ATTRACTANTS FOR FRUIT FLIES
(from literature and personal correspondence, 1978)

Investigator Number	Species	Colors		Shape	Sticky	Location in tree above ground
		Survey	Control			
5, 6	Anastrepha suspensa Caribbean fruit fly	arc yellow	-	15X20 cm	Stickum	1.75-2 m
26a	Anastrepha ludens Mexican fruit fly	yellow paint	-	?	?	?
3, 17	Ceratis capitata Mediterranean fruit fly	saturn yellow black	- -	15X20 cm sphere	Bird tanglefoot	?
3	Dacus cucurbitae Melon fly	aluminum saturn yellow	- -	15X20	" "	?
3	Dacus dorsalis Oriental fruit fly	saturn yellow	-	15X20	"	?
3, 9, 4	Dacus oleae Olive fly	saturn yellow	saturn yellow	15X20	"	top, middle, lower
3	Dacus tryoni Australian fruit fly	saturn yellow	-	"	"	-
1, 25, 12	Rhagletis cerasi European cherry fruit fly	saturn yellow	saturn yellow	"	"	3 meters
15	Rhagoletis cingulata Eastern cherry fruit fly	saturn yellow	saturn yellow	"	"	
19	Rhagoletis fausta Black cherry fruit fly	saturn yellow	?	"	"	-
20	Rhagoletis mendax Blueberry fruit fly	saturn yellow	saturn yellow	7.5 sphere	"	1-1.8 m
16, 10, 14	Rhagoletis pomonella Apple maggot fly	saturn yellow	dark red	15X20 cm sphere 7.5 cm	"	2-3 m

(Agee, 1978)

GENETIC SEXING TECHNIQUE : INTRODUCTION

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Abstract

The aim of genetic sexing in the context of pest control is normally to produce males without any females. Techniques can be prezygotic or postzygotic. The former catagory includes meiotic drive genes and autosomal sex determining genes, the latter includes temperature-sensitive sex-linked lethals, inherited sexual dimorphism and male-linked insecticide resistance. Prezygotic techniques enjoy the advantage of causing no wastage of zygotes although they may be associated with a reduction in male fertility.

Postzygotic techniques should ideally be applied as early as possible in the life history, preferably in the eggs, to avoid wastage. Prezygotic techniques may prove useful for particular species but postzygotic ones probably have the widest applicability.

GENETIC ASPECTS OF QUALITY CONTROL IN MASS-REARED INSECTS

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Abstract

1. The artificially-bred intended for release must be productive in the laboratory yet successful also when released into the wild. From the first generation of colonisation, selection will act in the direction of adaptation to the artificial environment. The need to ensure that this laboratory-adapted genotype will be successful under field conditions is central to the practice of quality control.

There are good reasons for believing that the less a genotype is genetically modified through its stay in the laboratory, the better it will compete in the wild under natural conditions. Yet it must also be borne in mind that, in certain cases, the phenotype best able to achieve control may be one in which performance surpasses the wild type.

The geneticist is therefore required to consider two matters, (1) ways of maintaining, as near as possible, a qualitatively and quantitatively "natural genotype" in laboratory colonies (2) the possibility of improving upon nature when necessary.

2. Genetic changes in laboratory colonies, occurring unintentionally, may be due to selection, including the influence of pleiotropism and linkage, or to random genetic drift, with the associated effect of inbreeding depression.

Phenotypes affected by selection include aspects of mating and oviposition behaviour, flight activity and possibly reproductive strategy. Selection often leads to a reduction in genetic heterogeneity in the more uniform laboratory environment. It may also alter the balance between genetic and non-genetic variance, and between additive and non-additive variance. There is probably no escaping selection in laboratory colonies although it may be possible to restrict it or direct it.

In theory, random genetic drift and inbreeding can be avoided if founder populations are sampled from large "central" field populations and laboratory colonies are kept large enough without "bottlenecks". In practice a number of uncertainties arise not least of which is the problem of defining a central population and sampling it over its full range of spatial and temporal variation. Attention also needs to be focused on the fact that the "effective population size" of a laboratory colony may be considerably smaller than would appear because only a small proportion of the total colony may become parents under crowded conditions.

Inbreeding has been known to lead to a number of undesirable consequences including a loss of fertility, reduced life span, lower mating frequency and loss of pheromones. Commonly associated with these changes are morphological and chromosomal abnormalities.

3. The genetic structure of laboratory colonies may be monitored by studying discontinuous phenotypic variation, by observing chromosomes or by estimating heritabilities. The best discontinuous phenotypes to study are those closest to the primary action of genes i.e. proteins, which are most easily monitored by electrophoresis. Directional changes in individual proteins have been observed although it is difficult to know their significance in terms of field performance. However the overall reduction in variation observed in many laboratory colonies is almost certainly disadvantageous.

Chromosomal changes can prove detrimental to fitness and are sensitive indicators of evolutionary change. Inversions are sometimes lost in laboratory colonies, a process which can be associated with an increase in lethals. Useful information on chromosomes is derived from polytene studies or from Giemsa bands.

Heritability estimates may help to determine at a quantitative level whether genetic changes have occurred in the laboratory, in relation to traits such as flight activity or response to pheromones.

4. Precautions may be taken to maintain or regain a natural genetic structure in a colony. Apart from general precautions regarding the choice of foundation stock and the avoidance of laboratory mortality and bottlenecks, other courses of action (specific to different control methods) include (a) regular strain replacement from the wild (b) provision of a variable laboratory environment or quasi-natural conditions in field cages (c) crossing to wild type before release (d) crossing two laboratory lines before release.

Stocks reared for different purpose will require different treatments. These will be discussed. No precaution should be assumed effective on the basis of theoretical arguments alone. There is no substitute for testing the product under field conditions.

5. The possibility of selectivity changing relevant traits (host preference, mating ability, temperature tolerance etc.) has been proved on many occasions in a variety of different insects but not yet in relation to field releases. Perhaps it is with parasites that selection offers the best prospects for improvement. If one accepts that a well adapted parasite behaves prudently towards its host, it follows that the requirements for control (in cases where the parasite is not expected to become permanently established) may be quite different from the adaptive requirements. The same argument could apply also to predators.

The dangers of selection in producing adverse correlated effects are often pointed out. Yet the experience of animal breeders leads one to suppose that the problems can be largely avoided, particularly when selection programmes are approached in a strictly empirical way.

6. Priorities for genetic research on quality control.

(a) Systematic study of chromosomal, electrophoretic and other variation, both in wild populations and in laboratory colonies of pest insects, attention to be paid particularly to genetic mapping of linkage groups, Giemsa banding and polytene mapping where possible.

(b) Investigations of ways to reduce, delay or compensate for deleterious genetic changes resulting from colonisation.

(c) Attempts to improve on nature by judicious selection.

(The key fields of study are population genetics, formal genetics, biometrical genetics and cytogenetics. Investigations on *Drosophila* spp provide the principal model).

GENETIC SEXING TECHNIQUES BASED ON TRANSLOCATION OF INSECTICIDES RESISTANCE TO THE Y CHROMOSOME

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In *Anopheles gambiae* s.s., and *An. arabiensis* resistance to dieldrin is normally controlled by a semi-dominant autosomal gene. Resistant males of each species were irradiated and crossed and backcrossed to susceptible homozygote females. In each species it has been possible to select a family in which the gene for dieldrin resistance had been translocated on to the Y chromosome so that males are heterozygous for resistance and females are susceptible homozygotes. Thus the females can be selectively killed by dieldrin treatment at the first instar larval stage leaving the males unharmed to be reared and prepared for a release programme.

The extension of this method of female elimination to many other species is theoretically possible. However, consideration must be given to the availability of suitable insecticide resistance loci where accurate discrimination of the genotypes is possible, the availability of a suitable Y chromosome so that male linked translocations can be produced, the possibility of crossing-over between the resistance gene and the translocation and means of preventing it, and the desirability or undesirability of release of males carrying resistance genes.

MATING COMPETITIVENESS OF *Tetranychus urticae* (ACARI : *Tetranychidae*) MALES OF A LINE HOMOZYGOUS FOR A STRUCTURAL CHROMOSOME MUTATION

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1. Age dependent mating competitiveness of males of line 2, homozygous for a Structural Chromosome Mutation (S.C.M.)

Experiments on male mating competitiveness showed that males of line 2 have a compititiveness value, which is not significantly different ($0.5 > p > 0.1$) from that of males of the wild-type strain. Studies of mating competitiveness of X-ray irradiated males had revealed a significant decrease in competitiveness during ageing (Ann. Rep. Association EURATOM-ITAL, 1976). This raises problems when the released males are supposed to have a long term effect on the reduction of population growth of a multivoltine species with overlapping generations. Thus experiments on the effect of ageing on mating competitiveness specifically of males of S.C.M.-line n°2, were necessary. The design of these experiments has been described elsewhere (Entomol. exp. & appl., p. 182-191, 1977). The results are given in table 1. The following conclusions were drawn. While separate experiments on male mating competitiveness involving males of equal age of S.C.M. N°2 and of wild type males did not yield significant differences ($0.7 > p > 0.1$), the summation of all experiments involving competition among males of equal age did ($p = 0.027$). The χ^2-value increases with the age difference between S.C.M.-males and wild-type males suggesting that ageing of the S.C.M.-males might have a negative effect on male mating competitiveness. This trend is, however, far from significant.

Table 1 - Effect of age on mating competitiveness of males of a line homozygous for a Structural Chromosome Mutation (S.C.M.).
First 2 columns: age in days of S.C.M. and wild-type males respectively.
Third and fourth columns: number of females mated by wild type or S.C.M.-males respectively. Fifth and sixth columns: G-value (cf. Sokal & Rohlf, Biometry, 1969) for homogeneity among replicates. Seventh column: χ^2-values on differences between mating types with associated p-value in eighth column.
Competitiveness-value of the S.C.M. males in last column.

Age of males involved in mating competition (days)		Number of females mated to (sum of 15 replicates):		Replicated test of goodness of fit (df=14)		χ^2-test on difference in mating type frequencies χ^2	$\bar{P}$	$\bar{C}$
S.C.M.	w.t.	S.C.M. males	w.t. males	G_H	G_H			
1	1	69	54	14.927 n.s.		1.829	0.176	0.878
2	1	65	51		14.603 n.s.	1.690	0.194	0.870
2	2	59	49	14.500 n.s.		0.926	0.664	0.907
3	3	65	49	15.485 n.s.		2.246	0.134	0.867
3	1	72	52		16.534 n.s.	3.574	0.059	0.839
Experiments with equal ages pooled: G_H = 44.912 n.s. (df=44) G = 4.884 →							0.027	0.891

GENETIC ANALYSIS OF ACTIVITY AND SEXUAL BEHAVIOUR IN *Drosophila melanogaster*

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Fitness characters associated with mating success of male *D. melanogaster* are : short latency and duration of courtship, long copulation duration, and a high level of locomotor activity. Amount and speed of locomotor activity are under separate genetic control. Selection for activity such as would change the rates of dispersal of released insects is associated with a correlated reduction in mating success.

Male courtship contains several discrete behavioural elements, including wing vibration to produce acoustic stimuli known as courtship song. This serves both for species identification and as a stimulus to the female to mate. The results of genetic analysis of the song will be discussed, together with the heterotic effects of a major gene mutation which increases courtship success by increasing the amount of song stimulation.

Males differ in their readiness to court females in different physiological states. Their responses have separate genetic bases according to the relevant stimulus inputs which probably involve a pheromone and courtship rejection responses by the females. The courtship investment strategies of males differ between wild populations in different geographical regions, and the adaptive significance of this is probably related to the average level of receptivity of virgin females in the different populations.

CONCEPTS AND APPROACHES

E.F. BOLLER and D.L. CHAMBERS

1. What is Quality Control ?

Quality control is a relatively new aspect of mass production of insects despite the fact that concepts and techniques developed and applied by industrial manufacturers have been available for a considerable time. Indeed, the basic philosophy of quality control does not need to be invented and developed from point zero in our fruit fly rearing programs. It has already been defined and described in many comprehensive books written for industry. The basic principles do exist and require only adequate adaptation to biological processes. Persons responsible for the design and implementation of quality control in insectaries will find a wealth of useful information and stimulation in several textbooks, such as those of D.A. SIMMONS (1970) and A.G. ROBERTSON (1971). Most of the concepts and approaches presented in this chapter have been taken from these two books.

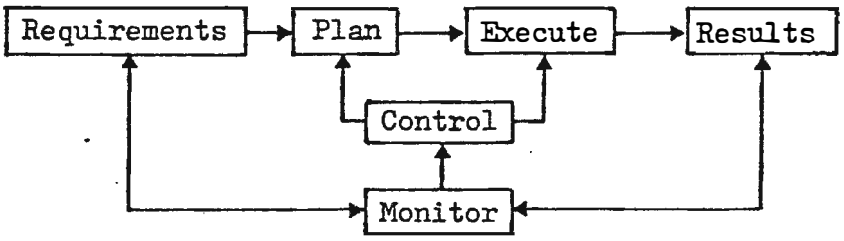
What is quality in the context of quality control ? The European Organization for Quality Control (EOQC) has defined quality as "... the degree to which a product meets the requirements of the customer". This market-oriented definition can be applied to mass reared insects when we substitute "objective or expected function" for the term "customer". The role of quality control is to provide and coordinate a production system that ensures that the operation will produce adequate numbers of an optimum quality at minimum product costs.

Two axioms reflect the direction the quality program should take : (1) Quality must be designed and built into the product, and (2) quality cannot be achieved by inspection (mere removal) of defective products).

Significant advances in industrial quality control were brought about during World War II when statistical quality control was established with its Shewhart control charts and other statistical tools. The present concept of total quality control was first introduced by FEIGENBAUM in 1961. The main difference between the total quality control concept and that of the early statistical era is that statistic is now a tool of the system and not the system itself (SIMONS, 1970). Total quality control utilizes a systems approach and emphasizes planning and measuring methods to ensure product quality rather than increased inspection (sorting good from bad). Quality control deals with the whole system of production and all methods that are used to establish and achieve standards. its aim is to identify the causes of deficiencies and to eliminate them by appropriate corrective action.

'It is with the aid of cybernetics, the science of control, that a better understanding of the nature of the processes involved can be achieved. The following simple diagram (KING, 1975) shows the nature of the feedback mechanism :

Fig. 1



2. The Basic Steps in Quality Planning and Implementation

There are several chronological steps that can be followed in any situation where quality control programs have to be established.

a. Define the objectives. Define for what purpose the insects are reared and identify the requirements.

b. Establish standards. The required attributes of the insects produced have to be specified (specification).

c. Design and test the production methods that satisfy the specifications.

d. Implement quality control to ensure, within the confidence limits required, that the end product conforms to the specifications (via monitoring and corrective action).

2.1. Objectives

Fruit flies are mass produced for purposes that range from the culture of parasites upon a biomass of fly tissue to the induction of infertility in a target population by genetically altered strains. The characteristics required of the colonized fly are defined by the objectives that it must achieve. The strictest quality specifications can generally be assumed to be required in the latter case, where intraspecific action resulting in infertility in the important criterion for the definition of standards. For further discussion of this aspect we refer to the literature (BOLLER, 1973 ; BOLLER and CHAMBERS, 1977 ; CHAMBERS, 1975, 1977 ; HUETTEL, 1976 ; MacKAUER, 1972, 1976).

2.2. Standards

Quality can be quantified only by its measurement against standards, which are the basis for developing and applying quality control procedures. Too often standards if present, are vague and poorly defined, whereas they should be precise and descriptive of both the average and the range of acceptable performance levels. Precise definition requires precise information however, and often our knowledge of fruit fly behavior, ecology, and genetics is inadequate.

The standard of reference generally felt to be most suitable in programs of sterile release is the target population against which the sterile fly must act. However, an internal standard (CHAMBERS, 1975, 1977 ; HUETTEL, 1976) is an acceptable reference for the detection of variations in performance of colonies already deemed competent by more stringent standards. Thus, untreated samples from the colony may serve as internal standards for judging the effect of sterilization, diet, marking, etc.

Quantification of performance standards becomes more complex as the objectives of the program become more demanding. Furthermore, it becomes increasingly evident that overall quality must be dissected into numerous components that are amenable to numerical assessment. The development of a hierarchy of quality components may aid in developing measurable units and a model of such a hierarchy is shown in Fig. 2 (adapted from BOLLER and CHAMBERS, 1977).

The overall quality (HUETTEL, 1976) of a laboratory population is measured in terms of how well it functions in its intended role, e.g., how effectively it interacts with and impacts upon the target population. There is temptation to proceed with a release program and let success or failure assess the quality of the production fly, but the hazard is great and unnecessary. Additionally, the costs inherent in such programs dictate that they be conducted efficiently, and efficiency can be assessed only through quality assessment. Thus, procedures that evaluate overall performance (the top of the hierarchy shown in Fig. 2) need to be developed and applied, but they can be interpreted only through insights provided by analysis of more discrete quality components.

In Fig. 2 overall quality is divided into five major components covering adaptability, motility, orientation to habitat, sexual activities (courtship and mating), and reproduction. Such subdivision allows sorting of those activities that are most critical to success or subject to alteration. A challenge at this step is determination of the relative importance of each selected component. An attempt to weight the components in relation to the objective of production is shown in Fig. 3. Such a subjective generalization has limited value but does aid in identifying aspects deserving of more intensive effort. Thus, sexual activities deserve special attention in programs of genetic control.

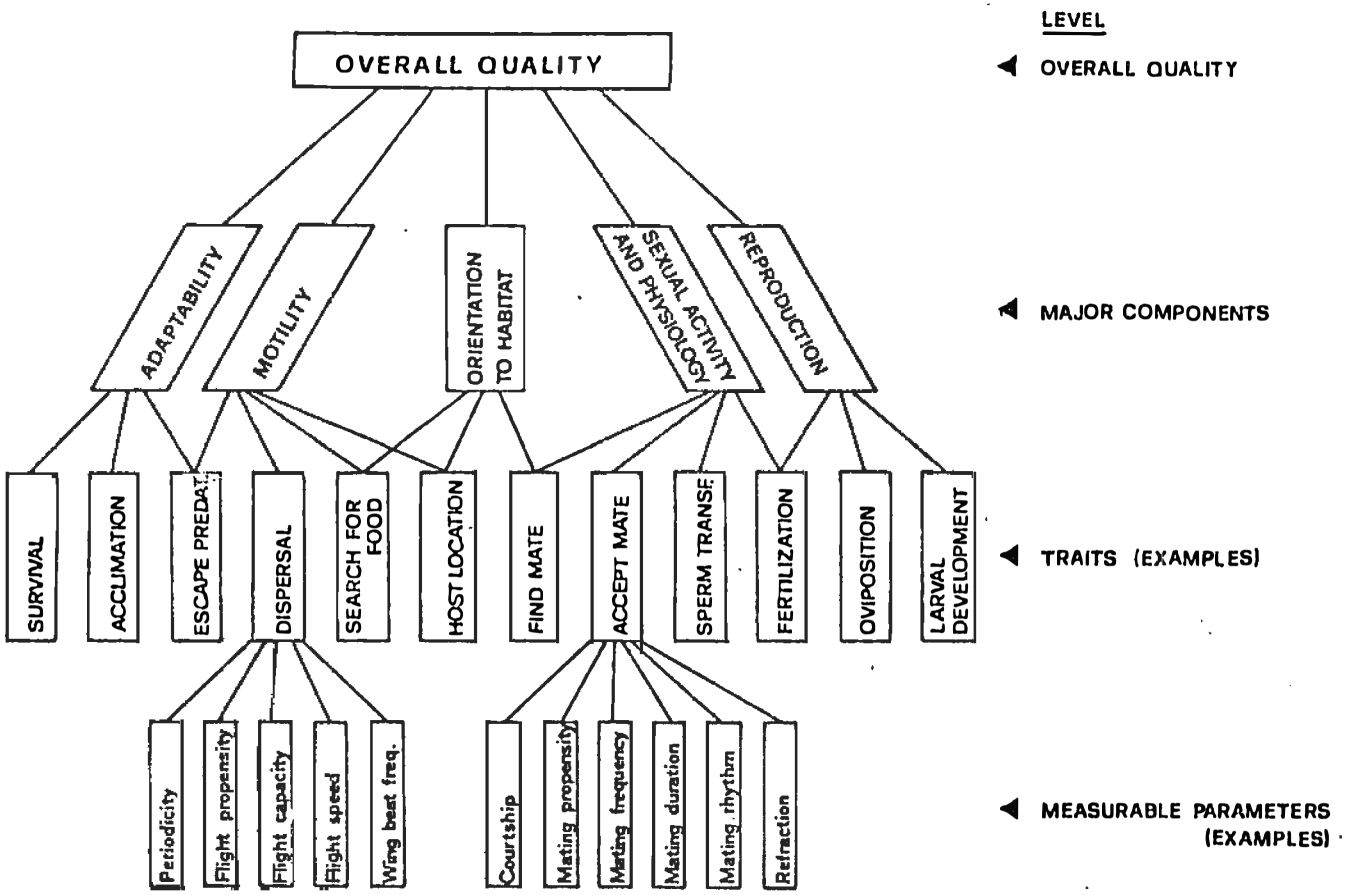


Fig. 2 Schematic presentation of a possible hierarchy of quality components with examples of second and third order interrelationships. (after Boller & Chambers 1977a)

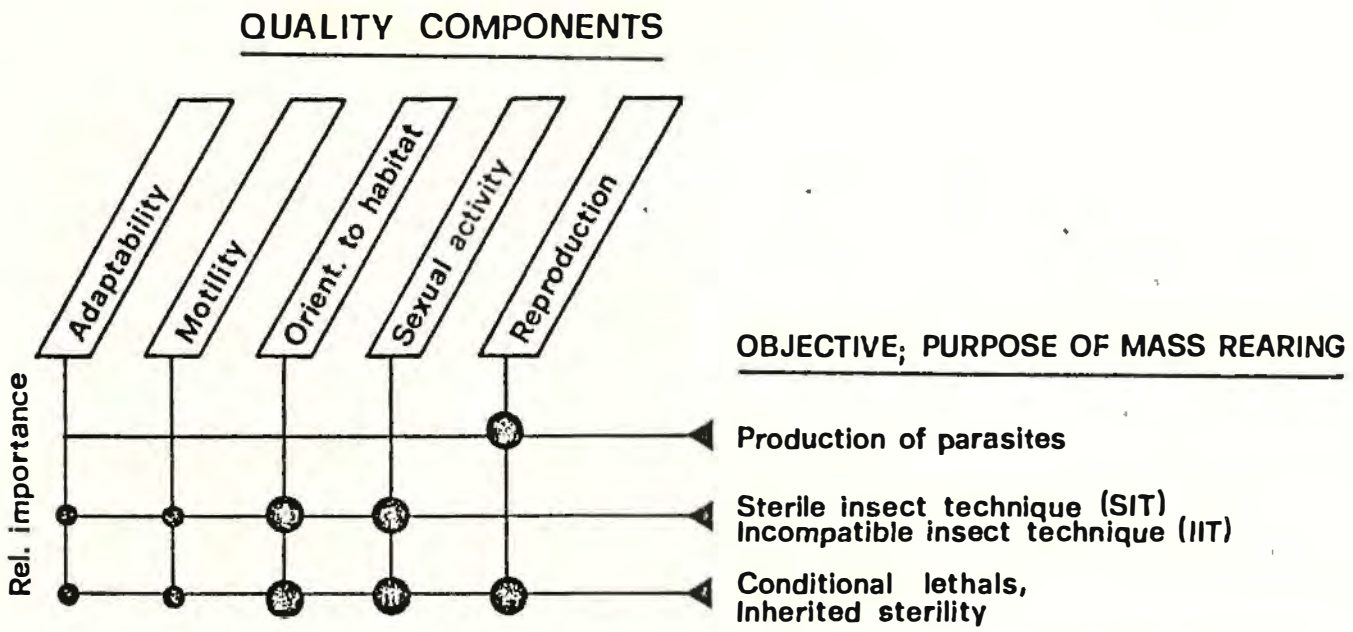


Fig. 3 Relative importance of major quality components according to the objectives of a rearing program (after Boller & Chambers 1977a).

Determination of the level of effort needed in assessing components determined to be of less than top priority may be more difficult than for those at the top. Thus, while normal dispersive behavior can be readily seen to be of value (COLUZZI 1971 ; BOLLER, 1972, BUSH et al., 1976), it may be that a realistic assessment of adequacy and/or logistical considerations will allow a standard lower than anticipated (CHAMBERS, 1977). Finally, certain quality components are irrelevant in establishing standards (e.g., colonizing properties in the field in release of sterile insects).

Determination of the major components allows one to proceed with somewhat more confidence in the selection of individual quality traits containing measurable parameters (Fig. 2). This level of the hierarchy shows, necessarily, a great variety of entries. Again, the listing should initially be as complete as possible and then reduced to those shown to have critical impact on overall performance.

2.3. Specifications : The problem of measuring and evaluation

"... when you can measure what you are speaking about, and express it in number you know something about it ; but when you cannot measure it, when you cannot express it in numbers, your knowledge, but you have scarcely, in your thoughts, advanced to the stage of science, whatever the matter may be" (Lord KELVIN, 1883). And, "... measurement is the comparison of an unknown with a standard" (ROBERTSON, 1971). And, "... quality in statistical quality control refers to some measurable property of the product that can somehow be translated into numbers" (SIMMONS, 1970).

Indeed, the establishment of specifications will call for an even further division of the traits into individual parameters amenable to direct measurement. One example is the subdivision of the trait embracing the activities involved in the location of the mating site, the sexual partner, or the host. Because both physical and chemical stimuli are involved in directing the insect to the proper site (such as color, odor, shape, or sound) different techniques are required to measure and interpret visual, olfactory and acoustical processes. This is the final level where a variety of techniques is to be developed for measuring and monitoring quality, where a wealth of data will be produced, and where it must be determined what techniques will produce information relevant for the events in the field.

The relatively complex structure of quality we have presented brings us into conflict with a need for quality control procedures that should be relatively simple in order to be applied widely and routinely. This breakdown of quality into innumerable parameters might indeed lead to the wrong conclusion that quality control is so complex and sophisticated that it becomes the privilege (or pleasure) of a few specialists. This is not the case. Thanks to the rapidly accumulating experience, improved and simplified methods and devices, and not

the least to the services provided by specialized facilities, entomologists should soon be able to analyse their problems and select or develop insects of the quality needed. The many techniques described in the larger part of this book reflect the increasing volume of ideas and options.

2.4. Adopting techniques from industry to insect production

Several interesting concepts and techniques have been developed for industrial quality control that merit the attention of entomologist's responsible for the design and implementation of quality control in insect production facilities. Again, the key elements are described in detail by SIMMONS and ROBERTSON, whose books might be studied with great profit. The following outline summarizes some of the salient features that may be adopted for our purposes.

In every process random variation is present. This variation is inherent but there is also induced variation that is directly attributable to specific causes. We often refer to these two types of variation as genetic and environmental variation ; both acttogether and produce what we call the phenotype, or phenotypic expression of a certain quality trait. In essence, statistical quality control investigates processes and locates and separates these two types of variation in order that meaningful steps can be taken to control "quality". When only inherent variation is at work in a process, we consider the process to be under control. The ranges of inherent (genetic) variation of a trait that follows a normal distribution are called the capability limits of the system, which cover six standard deviations (ROBERTSON, 1971). The 3 σ (sigma ; standard deviation) is a key element for the evaluation of the dynamics of a production process and also the basis for establishing the specification limits (Fig. 4). Capability limits and specification limits are also called, according to their functions, the control (warning) and action limits, respectively.

When we study wild insect strains using the techniques described in this book we are measuring phenotypic expressions of a given quality trait under a defined set of circumstances. The frequency distribution of the measured values is the central part of our investigation as it tells us essential characteristics of the trait. In industry this basic study is called the process capability study. Process capability is defined (as shown in Fig. 4) as the 6 σ range of a process under specified conditions. The calculation should be based on data from at least 50 samples. The data should be plotted to ensure the presence of a normal distribution and, where doubts exist, the data should be tested with statistical methods. For most applications the visual (or "eyeball") test of the plotted frequency distribution should tell the story. The results obtained in these capability studies are the basis for the design of quality control tests and evaluation techniques.

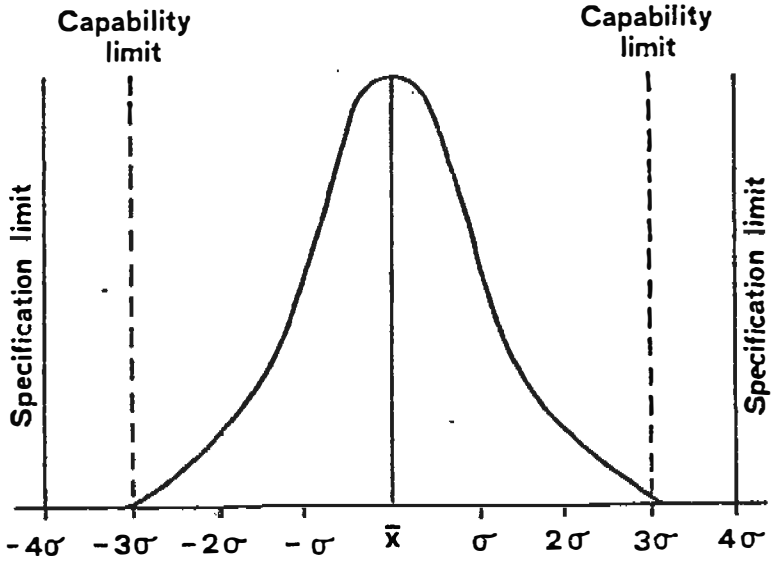


Fig. 4 Specification and capability limits of a quality trait.

Once the capability limits have been obtained we can proceed to the next important step, which leads to the establishment of the terms of reference in our quality control program, the specifications or tolerances of the trait with its upper and lower boundaries clearly defined. Specifications should always be realistic and, thus, as wide as possible consistent with satisfactory function of the insects produced. The larger the quantities being produced, the greater the need for wider tolerances and the bigger their impact on costs. When establishing specifications the first step is to separate the few vital ones from the many that rank lower on the priority list. It is a common practice in manufacture to set the specification limits for a range of 8 σ . Whether this practice can be applied to insects remains to be tested. Setting the specification limits tighter than necessary is an expensive exercise. Specifications must be feasible from a production and measurement point of view.

The Shewhart control charts were among the first statistical tools to be introduced in the era of statistical quality control. Their purpose is to plot a parameter with predetermined limits on a time scale and to present this information in an easy to interpret graphical form. By plotting sample results as averages ($\bar{X}$-chart) or ranges (R-chart) on a time scale, we can ascertain whether the variation from sample to sample is due to chance (random) variation or to assignable causes. The criteria used to make these decisions are the control limit lines (Fig. 5).

Control charts are a type of hypothesis testing. The hypothesis being tested is : Is the process "in control" ? In these tests two errors can be made :

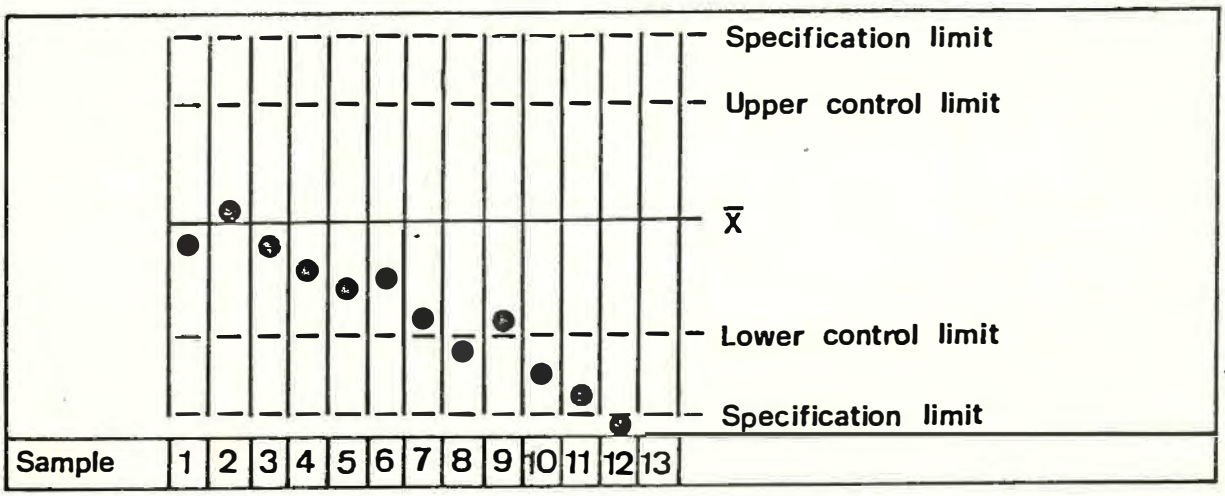


Fig. 5 Example of a quality control chart for averages ($\bar{X}$-chart)

1. If we reject the hypothesis that we are in control when in fact we are in control, we make an error (type I). The consequence is that we will look for nonexistent troubles, which will cause unnecessary efforts and costs.

2. If we concluded that the process is in control when in fact it is not, we make another error (type II). The consequence is that we do not look for trouble when it is present and thus continue to produce products that do not meet standards.

$\bar{X}$- and R-charts therefore provide information on three matters, all of which need to be known as a basis for appropriate corrective actions :

- Basic variability of the quality characteristics
- Consistency of performance
- Average level of performance of the quality traits

For further details on these techniques (sampling, rejection criteria, statistical background, etc.) we refer to the pertinent literature (e.g., GRANT and LEAVENWORTH, 1972 ; KING, 1975; OTT, 1975 ; ROBERTSON, 1971 ; SIMMONS, 1970).

3. Organizational aspects of quality control

Most industrial enterprises have quality control groups or units. These do not exist in comparable form in most insect rearing facilities. However, certain aspects of quality control organization are of general validity and could be adopted accordingly.

All units or individuals responsible for quality control should have well defined responsibilities and authority and should have the organizational freedom to identify and evaluate quality problems and to initiate, recommend, and provide solutions. These points will deserve greater attention wherever quality control and production are not in the hands of the same persons (e.g., in large rearing facilities). Major problems are likely to arise when the operation succumbs to the pressures of shipping schedules. If an adequate and acceptable procedure is not established and agreed upon by quality control personnel, project managements, and personnel of the production line, the quality control program can degenerate into periodic end-of-the-month activities involving constant reworking or continual changing of tolerances and lowering of standards. When quality has been adequately defined and built into the product by careful preparation then the production flow should be smooth with substandard insects at a minimum and the need for concessions and modifications at a low level.

The postulated form of the relationship between quality control and feedback processes used in total quality control (cf., ROBERTSON, 1971) is shown in the following flow diagram and should be the aim of any organization that wants to achieve the most cost-effective method of producing items that meet a specified quality.

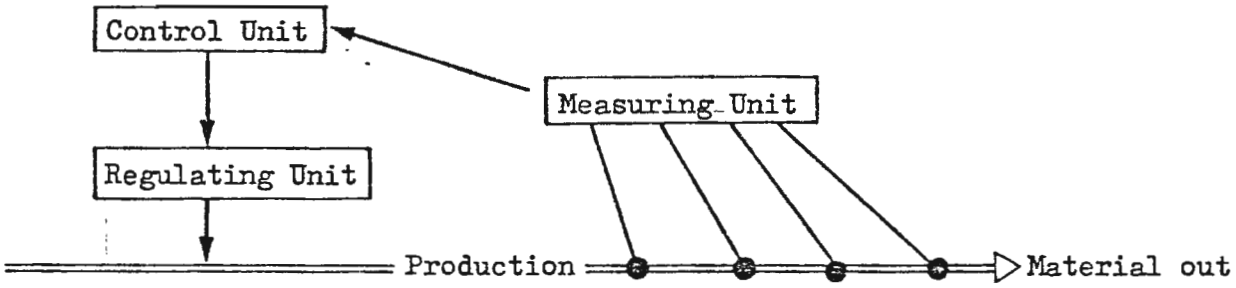


Fig. 6

4. The economics of quality

The economic aspect of quality control has never been analyzed in conjunction with insect production and there are no figures available that could support that analysis. The economics of quality might, however, become a major point of interest with increasing capacities of the rearing plants and need for their efficient operation. Although figures given by industry must be interpreted with great care when extrapolated to governmentally operated insect production plants it might be of interest to examine the order of magnitude of estimations made in the United Kingdom (ROBERTSON, 1971). Quality and reliability costs of mass-produced products fall into three categories : prevention costs, appraisal costs (when production gets out of control) and actual failure costs. The distribution of these three components under systems emphasizing product checks has been estimated as follows :

failure costs	65 %	present average costs for quality = 4-20 % of gross turnover
appraisal costs	30 %	
prevention costs	5 %	

Potential costs under systems implementing total quality control with emphasis on prevention :

prevention costs	10 %	Savings might range from 1.5 to 6.5 % of gross turnover
appraisal costs	20 %	
failure costs	35 %	
savings	35 %	

5. Concluding remarks

Quality control in mass reared insects : In what direction is it heading ? Which of the present problems call for intensified investigation ?

One thing has become evident. We force our fruit flies through a series of genetic bottlenecks as soon as we bring the wild material to the laboratory for mass production under artificial laboratory conditions. Two major bottlenecks often occur when all life stages are reared exclusively on artificial substrates : one that occurs in the adult stage reduces drastically the reproducing portion of the founder population. The second one occurs during the larval stage, and its effect is often more conspicious because the pupal yield in the early phase of mass rearing is usually very low. Mortality occurring during the larval stage will eliminate those individuals that cannot survive on the new diet and selection will favor those exhibiting the appropriate physiological disposition. Its effect on important behavioral traits will not appear to be dramatic, as it will influence mostly characteristics that rank low in the quality hierarchy outlined in Fig. 2. However, its impact on the numbers of insects produced (also a quality criterion) may be severe. The impact of bottlenekcs that affect the adult stage are more severely felt by performance criteria, as such selection acts directly on those characteristics of adult behavior that have an immediate relationship with performance quality. Most of the techniques applied in fruit fly laboratories and described in this book deal with adult behavior. There is increasing evidence that this is justified.

May opportunities remain to advance the state-of-the-art. These advances will probably occur in techniques, methods, and programs rather than in quality control philosophy. The lack of good measuring tools and the lack of suitable techniques to evaluate quality data has hampered our ability to demonstrate the effectiveness and value of quality control programs. The many bits and pieces of information and ideas contributed by as many fruit fly workers all over the world have found their first precipitation in this book. The idea book by itself will certainly not completely solve the problem of designing and implementing the quality control programs urgently needed in our fruit fly facilities. We hope, however, that it will stimulate discussion and initiate a fruitful exchange of ideas

between entomologists working in theoretical and applied fields, resulting in further research, development and trial implementations. The book may also help to clarify the open questions and indicate areas of future actions.

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RECOMMENDATIONS

1. After reviewing the work of the different groups and bearing in mind the need to apply these results immediately in the context of present pest management practices it was felt that a change in organization was necessary to make this process more efficient. The following scheme for restructuring the fruit fly groups is suggested to the Council :

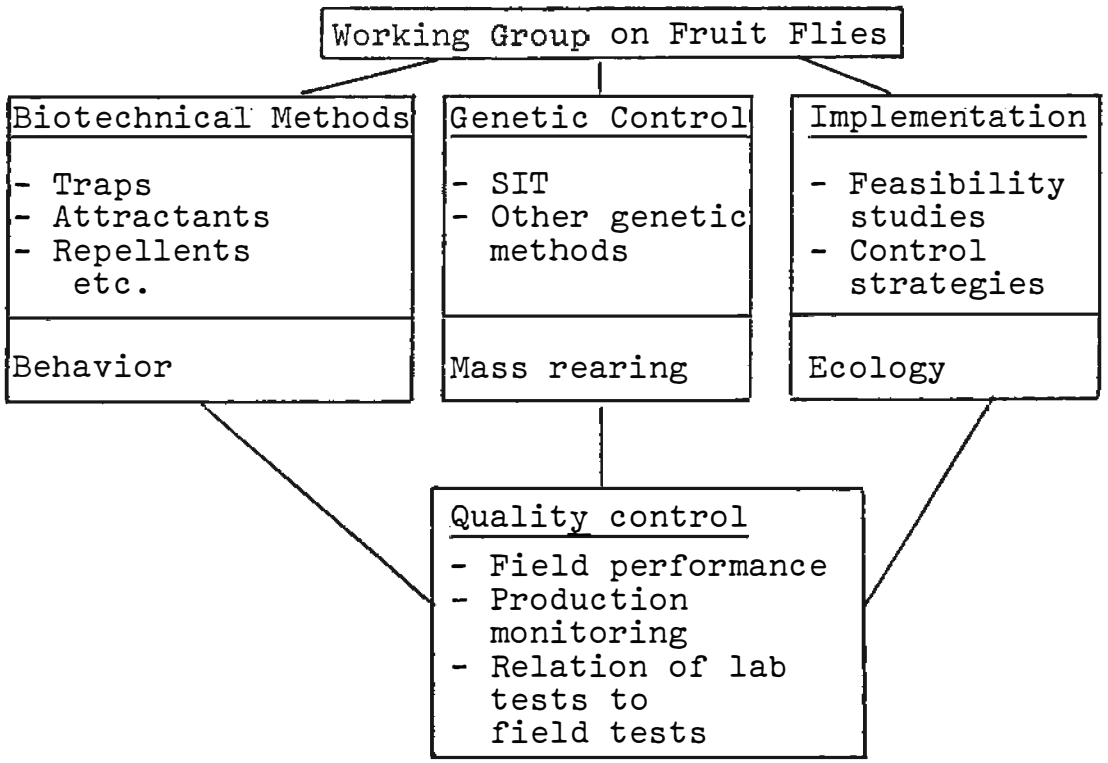
1.1. Working Group on Integrated Control of Olive Pests

Convenor : Dr. U. CIRIO, Italy

The structure and activity program of this group will be submitted to the Council by the convenor.

1.2. Working Group on Fruit Flies

This new group will replace the existing Working Groups on Genetic Control of *Ceratitis capitata* and *Rhagoletis cerasi*. Proposed structure :



In the first two sub-groups new ideas and methods will be developed ; in the third sub-group these developments will be applied immediately in practice. The quality control unit supports the three sub-groups in their work and can assist other working groups in solving quality problems occurring in mass rearing of other insects.

The activity program for immediate coordinated research is given in Appendix I.

2. The Working Group on Genetic Methods of Pest Control recognizes *Ceratitis capitata* as a high priority insect and is very interested in carrying out genetic studies on this insect, particularly on behavior genetics, on genetic variation in relation to environmental variability, and on developing genetic and cytological maps. Such work is warmly welcomed by the Working Group on Genetic Control in *Ceratitis capitata*.

To make such investigations possible we recommend further contacts between members of the Working Group on Genetical Methods and of the proposed new Working Group on Fruit Flies.

- to encourage young scientists to spend time in the laboratories of members of the Working Group on Genetical Methods to carry out genetic studies on *Ceratitis capitata* in relation to quality control and to the development of a genetic sexing technique.

- to provide opportunities for future meetings between the proposed new Working Group on Fruit Flies and the Working Group on Genetical Methods whenever such joint meetings are deemed desirable by the convenors.

3. Considering the importance of the Joint Mexican-USA Program on *Ceratitis capitata* now in progress, it is recommended that regular contacts be developed between that program and the proposed Working Group on Fruit Flies. This could be achieved most efficiently through an individual designated for this purpose by the Council.

4. The working groups have examined the present situation of pest control in Sardinia and strongly support the following resolution :

Considering that the economical development of Sardinia in the agricultural field is oriented more and more towards the increase of fruit plantations and in consideration of the eco-ethological knowledge acquired recently in the field of fruit flies species being a hazard to the main fruit crops in Sardinia, and following above all the intentions and recommendations made by the OILB Working Group on *Ceratitis capitata* at its first meeting in Sardinia (1975), we propose the development of a concrete program in Sardinia with short term and long term objectives.

Based on the presently available information and knowledge we suggest to initiate experimental activities with the objective to develop an integrated control program for the main fruit pests and aiming at a reduction of the application of polluting pesticides.

We recommend to expand the existing investigations on the biology and population dynamics of the pest species in order to develop more efficient and specific pest management systems in Sardinia.

The Regional Administration of Sardinia is invited by the OILB working groups that held their joint meeting in Sassari to prepare the necessary infrastructures for field operations and for technical assistance that form the basis for program implementation. Also it is emphasized that the future success will largely depend on close contacts between scientific institutions and agricultural operators.

During the first phase of realization of such a program we recognize the need for a close cooperation at the national and international level and for a continuing technical-scientific assistance during program development and implementation. The implementation of such a program is not only of interest to Sardinia but also to other Mediterranean regions that could join the program and could profit of the technical and financial support from international organizations (such as the Commission of the European Community, FAO, IAEA and OEPP).

(The Italian translation of this resolution is given in Appendix II).

APPENDIX I

Proposed co-ordinated research program (1978)

Until the Council has approved the proposed new structure of the fruit fly groups the individual working groups will continue their internal activity programs that will be submitted to the Council by the respective convenors. However, it was decided to initiate immediately two international research programs that require the cooperation of specialists working on *Ceratitis capitata* and *Dacus oleae*.

1. International research program for the development of efficient visual traps

This is a joint program between the existing OILB fruit fly groups and the USDA Insect Attractants, Behavior and Basic Biology Research Laboratory at Gainesville, Florida, USA (Dr D.L. CHAMBERS, director). The objective of this program is the measurement of the spectral sensitivity of *Ceratitis capitata* (top priority), and *Dacus oleae* by US specialists in the fruit fly laboratory at Wädenswil, Switzerland, and to correlate these electrophysiological data to field data obtained in various European regions by coordinated and standardized color experiments (color traps).

The principal scientific investigator is Dr.H.R. AGEE (USDA, Gainesville). He is assisted by Dr. E.F. BOLLER (Switzerland) who coordinates the respective activities in Europe.

The following specialists have agreed to participate and will receive detailed information in the near future :

1.1. *Ceratitis capitata* (top priority)

Drs DelRIO, Sardinia ; FIMIANI, continental Italy ; ROS, Canary Islands and continental Spain ; NEUENSCHWANDER, Crete ; GALUN, Israel.

1.2. *Dacus oleae*

Drs SILVA, Portugal ; CIRIO, continental Italy ; NEUENSCHWANDER, Crete ; HAMEIRI, Israel.

Whenever there is sufficient capacity for the spectral analysis the following colleagues have announced their interest and will be invited to participate : Drs ALVARADO, Spain; ARAMBOURG, France ; ECONOMOPOULOS, Greece ; DEUSE, Italy.

2. Request for assistance in the world survey on genetic variability of *Ceratitis capitata*

It was agreed to support the request of Dr CHAMBERS (USDA) for cooperation in a world-wide collection campaign for

Ceratitis capitata pupae to be analyzed in Gainesville in the context of the Mexican-US program.

Dr E.F. BOLLER (Switzerland) agreed to coordinate the respective activities in Europe and Will provide detailed information to all medfly specialists in the near future.

APPENDIX II

Proposed resolution

Tenuto conto che lo sviluppo economico della Sardegna in campo agricolo è sempre più orientato verso l'incremento delle colture frutticole ed in considerazione delle conoscenze eco-etologiche acquisite recentemente sui ditteri Tripetidi dannosi alle principali coltivazioni, a seguito soprattutto delle intenzioni e delle raccomandazioni emerse dal Gruppo di Lavoro OILB sulla *Ceratitis capitata* riunitosi a Sassari nel 1975, si propone lo sviluppo di un programma concreto con obiettivi a breve ed a lungo termine.

Sulla scorta dei dati attualmente disponibili si ravvisa l'opportunità d'intraprendere una serie di azioni di estensione sperimentale rivolte principalmente alla realizzazione di una lotta integrata a favore delle principali colture da frutto con l'obiettivo di effettuare interventi più tempestivi e ridurre nel contempo l'applicazione di prodotti tossici inquinanti.

Per giungere a forme di intervento più risolutive e specifiche e per applicazioni su superfici sempre più ampie, si ravvisa la necessità di approfondire ed estendere gli studi biologici e di dinamica di popolazione delle specie novice già intrapresi.

L'Amministrazione regionale viene invitata dai Gruppi di lavoro dell'OILB riunitisi a Sassari ad apprestare le indispensabili infrastrutture operative e di assistenza tecnica che rappresentano la base per l'attuazione di programmi la cui riuscita è subordinata alla esistenza di stretti contatti tra struttura scientifica ed operatori agricoli.

Nella fase di prima realizzazione si avverte la necessità di una cooperazione a livello nazionale ed internazionale e di assistenza tecnico-scientifica anche durante lo svolgimento del programma.

L'attuazione di siffatto programma per l'interesse che potrebbe suscitare nell'ambito di tutta l'area mediterranea, dovrebbe avvalersi del contributo finanziario di Organismi internazionali (quali la Commissione della Comunità Europea, FAO, IAEA, OEPP.

ANATOMICAL, HISTOLOGICAL, ULTRASTRUCTURAL AND PHYSIOLOGICAL STUDY OF THE RECTUM AND ETHOLOGICAL OBSERVATIONS IN RELATION TO THE HYPOTHETICAL SEX PHEROMONE PRODUCTION BY THE MALE OF *Dacus oleae* GMEL.

DE MARZO L., NUZZACI G., SOLINAS M.

Istituto di Entomologia Agraria dell'Università di Bari

SUMMARY (*)

The production and storage of a sex pheromone in glands associated with the rectum, described for the first time by FLETCHER (1968) in the male of *Dacus tryoni* (FROGGATT), has been hypothesized (SCHULTZ and BUSH, 1971 ; ECONOMOPOULOS, GIANNAKAKIS, TZANAKAKIS, VOYADJOGLOU, 1971) also for *Dacus oleae* Gmel. This paper proves that the said hypothesis is correct on anatomical, histological, ultrastructural and physiological ground, together with complementary ethological observations. In particular, the results of the anatomical, histological, ultrastructural and physiological observations on *D. oleae* rectum bring to light :
i) a functional organization of the rectal muscular coat, particularly suitable for accomplishing gradual (intermittent) but vigorous expulsion of some substances, that (like sex pheromones) must be diffused as widely as possible in the environment air ;
ii) four morphologically and physiologically different types of epithelium : a typical absorbent epithelium, from the rectal valve to the opening of the rectal ampulla ; an epithelium "of transition", among the bases of the rectal papilliae ; a squamous epithelium, in the fore third (except the opening) and along a dorsal strip of the rectal ampulla, and in the anal tube ; and a typical glandular epithelium, which lines the mid and hind parts of the rectal ampulla. In sexually immature males, the glandular epithelium secretes an "oily substance" without any odor, while, in sexually mature males, the said epithelium secretes, together with the "oily substance", other substances with an odor (vaguely ammoniacal) well-known as "sex odor" of the *D. oleae* males.

The ethological observations, particularly the olfactometric ones, prove that *D. oleae* males strongly attract females by a sex pheromone, at the time of the day when the species is sexually active.

(*) The paper is in print in "ENTOMOLOGICA", Bari, vol. XIV, 1978, pp. 150-213, 40 figs, 38 refs.

Critical comparison and integration of the results obtained thus far clearly show that *D. oleae* male "sex odor" is the same as the sex pheromone, and that the "oily substance" may act as a carrier for this very much volatile pheromone.

Dacus oleae Gmelin RESPONSE TO VARIATIONS OF ABIOTIC FACTORS

Vincenzo GIROLAMI

Istituto di Entomologia agraria dell'Università di Padova

Available experimental data (and, whenever possible, relative equations) on the following topics concerning *Dacus oleae* Gmelin are shortly discussed :

1) Survival and development lenght of juvenile stages with temperatures included between -10°C and 45°C (at 2.5°C intervals), and adult behaviour at the same temperatures and survival without food.

2) Fecundity and survival of adults for temperatures included between 18°C and 38°C (intervals ≤ to 2.5°C) which are steady up to 25°C and also alternating from 25°C to the higher values. Findings concerning the influence on fecundity, of nutrition, relative humidity, light intensity, conditions of juvenile growth, availability of egg-laying beds.

3) Findings concerning the influence of temperature, acclimatisation at low thermic values, nutrition, air movement, light intensity on the insect flight.

Investigations have been carried out in the field, in temperature controlled chambers (± 0.25°C), in a green house (automatically controlled and shaded : ± 2°C) also inside plexiglass boxes (± 0.5°C).

The thermic values refer to the body temperature of insects, the respective prejudicial measuring methods will be discussed.

THE INFLUENCE OF THE INFESTATION FROM *Dacus oleae* Gmel. ON THE QUANTITY AND ON THE QUALITY OF THE OLIVES PRODUCTION IN UMBRIA

Claudio PUCCI, Salvatore CECCARELLI e Bixio FILIPPUCCI

Istituto di Entomologia Agraria. Istituto di Allevamento Vegetale della Universita degli Studi di Perugia e Istituto Sperimentale per l'Olivicoltura di Spoleto

SUMMARY

In this paper the results of an experiment designed to study the influence of the level of the infestation (from *Dacus oleae*) on the quantity and on the quality of the oil are described. As experimental material the olives on the plants of three varieties ("Dolce Agogia", "Rosciola" and "Moraiolo") have been used to give 11 samples at different levels of infestation. The data of this experiment should give, together with those not yet analyzed on the fruit drop, an information on the economic threshold.

The results obtained show clearly that the relationship between oil acidity and level of infestation is linear in "Dolce Agogia" and "Moraiolo" and it is described by the equation of a parabola in the variety "Rosciola".

In Table 1 the observed and expected values on the basis of the specific equation for each variety are reported.

As far as the oil vield is concerned, its relationship with the level of infestation is much more complex : however the straight-line equation appears to give a good agreement between observed and expected values (shown in Tab. 2) and therefore appears to have good previsional value.

This research has been supported by the Italian National Research Council. Special "ad hoc" program "Fitofarmaci e Fitoregolatori" Subproject n. 2.

Tab. 1 - The observed and expected values of acidity degree of three varieties in relation to the level of infestation.

Infestation %	Dolce Agogia		Rosciola		Moraiolo	
	Observed Values	Expected Values $Y = 0.31465 + 0.011613 X$	Observed Values	Expected Values $Y = 0.552859 + 0.0013296 X + 0.000162 31 X^2$	Observed Values	Expected Values $Y = 0.4316 + 0.013814 X$
0	0.34	0.31	0.50	0.55	0.42	0.43
10	0.47	0.43	0.61	0.58	0.62	0.57
20	0.59	0.55	0.66	0.64	0.80	0.71
30	0.68	0.66	0.80	0.74	0.86	0.85
40	0.78	0.78	0.88	0.87	0.96	0.99
50	0.88	0.90	1.01	1.03	1.02	1.13
60	0.96	1.01	1.14	1.22	1.13	1.26
70	1.02	1.13	1.48	1.44	1.40	1.40
80	1.08	1.24	1.66	1.70	1.61	1.54
90	1.27	1.36	1.96	1.99	1.67	1.68
100	1.79	1.48	2.36	2.31	1.89	1.82

Tab. 2 - The observed and expected values of oil yield of three varieties in relation to the level of infestation.

Infestation %	Dolce Agogia		Rosciola		Moraiolo	
	Observed Values	Expected Values $Y = 20.71 - 0.0324 X$	Observed Values	Expected Values $Y = 30.95 - 0.0338 X$	Observed Values	Expected Values $Y = 20.94 - 0.0318 X$
0	20.7	20.7	32.4	30.9	21.1	20.9
10	20.1	20.4	30.2	30.6	20.5	20.6
20	19.8	20.1	29.7	30.2	20.3	20.3
30	19.8	19.7	29.6	29.9	20.0	20.0
40	19.6	19.4	29.0	29.5	19.7	19.7
50	19.3	19.1	28.9	29.2	19.3	19.4
60	19.0	18.8	28.7	28.8	19.1	19.0
70	18.7	18.4	28.5	28.4	18.9	18.7
80	18.2	18.1	28.2	28.1	18.4	18.4
90	17.7	17.8	28.0	27.7	18.2	18.1
100	17.0	17.5	27.6	27.4	17.6	17.8

ADULT CAPTURES OF *Dacus oleae* Gmel. BY PROKOBOLL TRAPS IN UMBRIA

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SUMMARY

In this paper the results of an experiment carried out in 1977/78 on population dynamic of *Dacus oleae* Gmel. are presented ; the populations have been monitored capturing the adults with "Prokoboll" traps on five varieties of olive tree at each of three location are discussed. The factorial design of the experiment and the evaluation of a number of plants of each variety allowed the study of the effect of main factors (variety, plants within variety, date of capture, side of the traps and sex) as well as of the interactions between factors.

The results show (Tab. 1) that the variability between the average number of flies, males and females, captured on individual plants is significant in all the three locations : only in one location (Castel Rigone) the variability between variety was significantly higher than the variability between plants within variety. The variability between date of capture has been also always significant.

In all the three locations the following two-factor interactions have been significant :

1) plants within variety by sex ;
2) plants within variety by side of the trap ;
3) date of capture by sex.

The significance of the first two interactions shows that both the difference between the average number of males and females captured and between the average number of adults captured on the two sides of the trap change from one single plant to another.

The significance of the date of capture by sex interaction shows that the males/females ratio changes over time ; this explain why there is no difference between the averange number of males and females captured over the entire period.

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In Tables 2 and 3 the average number of adults captured on the five varieties and in the three locations is reproted : in particular in Table 1 is reported the average number of males and females and in Table 2 the average number of adults captured on the inside (1) and on the off-side (0) of the trap. It is remarkable that the males/females ratio varies both in different varieties and in different locations. Table 2 shows that the average number of adults captured on the inside of the traps is always higher than that captured on the off-side though the difference is not always significant.

The following three-factor interactions were also significant in all the three locations :

1) date of capture by side of the trap by sex ;
2) plants within variety by date of capture by side of the trap.

This means that the difference between the average number males and females captured on the inside and on the off-side of the trap changes in relation with the date of capture and that the difference between the average number of adults captured on the two sides of the trap changes both with the date of capture and with the single plant considered.

It appears interesting to note that the significance of some three-factor interactions may be an indication that the population dynamic of *Dacus oleae* Gmel. is very complex and that for further studies may be advisable to examine larger samples of plants for each variety.

In conclusion the data collected in 1977/1978 show that :

1) the number of adults captured varies more between the single plants than between varieties ;

2) the number of adults captured on the inside is always higher than that captured on the off-side of the trap ;

3) the male/female ratio changes both with the single plants and with the date of capture ; therefore any conclusion on the composition of the population of *Dacus oleae* Gmel. drawn from a limited number of captures date and of plants can be only of a limited value.

Tab. 1. – Results of the analysis of variance of the number of adults captured on "Prokoboll" yellow traps on different varieties of olives trees at different locations of Umbria.

Source of variation	D.F.	Mean squares		D.F.	Mean squares
		Castel Rigone	Passignano		Tuoro
Variety (V)	4	1.703,2 *	214.6	3	367.1
Plants within variety (P_e)	11	468.5 **	207.8 **	11	188.0 **
Time of sampling (T)	32	5.296.1 **	1.469.3 **	32	772.5 **
Side of traps (S_t)	1	6.584.0 *	1.995.6 **	1	194.1
Sex (S)	1	1.127.3	122.6	1	442.5
V x T	128	250.8**	44.8	96	60.6 **
V x S_t	4	259.9	58.1	3	14.6
V x S	4	586.1*	58.2	3	20.6
P x T	352	130.0	45.6*	352	30.7
P_e x S_t	11	1.130.0**	90.3 **	11	58.2 **
P_e x S	11	125.5 **	17.8 **	11	19.0 *
T x S_t	32	668.6	204.4 **	32	109.1 **
T x S	32	279.1 **	31.9 **	32	60.9 **
S_t x S	1	599.3	68.7 *	1	29.6 *
V x T x S_t	128	84.5	22.9	96	16.4
V x T x S	128	103.7 **	10.9	96	11.4 **
V x S_t x S	4	187.2	43.4	3	13.2
T x S_t x S	32	142.8 **	19.1 **	32	12.2 **
P_e x T x S_t	352	112.6 **	33.2 **	352	25.0 **
P_e x T x S	352	49.6	11.5 **	352	6.2
P_e x S_t x S	11	71.7	14.1 **	11	7.8
Error	480	48.7	4.0	448	6.0
Total	2.111			1.979	

* P 0.05
** P 0.01

Tab. 2 - Average number of adults of Dacus oleae Gmel. (♂ and ♀) captured per plant from 11.7.1977 to 27.2.1978 on five varieties of olive tree at three locations of Umbria.

Variety	Castel Rigone		Passignano		Tuoro	
	♂	♀	♂	♀	♂	♀
Dolce Agogia	7.9	4.9	3.8	3.3	3.1	2.0
Moraiolo	3.7	4.3	2.5	2.3	1.1	0.8
Frantoio	5.6	5.2	2.6	2.8	2.2	1.3
Rosciola	3.0	2.3	4.7	3.0	3.8	2.5
X *	13.8	6.8	1.5	1.9	-	-
L.S.D.	2.4		0.9		0.9	

Tab. 3 - Average number of adults of Dacus oleae Gmel. captured on the inside (I) and on the offside (O) of the "Prokoboll" traps from 11.7.77 to 27.2.78 on five varieties of olive tree at three locations of Umbria.

Variety	Castel Rigone		Passignano		Tuoro	
	O	I	O	I	O	I
Dolce Agogia	4.3	8.6	2.6	4.4	2.1	2.9
Moraiolo	3.2	4.7	1.5	3.3	0.9	1.0
Frantoio	3.3	7.6	2.2	3.2	1.5	2.1
Rosciola	2.0	4.3	2.2	5.4	2.7	3.6
X *	6.1	13.7	0.7	2.7	-	-
L.S.D. 0.05	2.4		2.2		1.5	

* The variety indicated by X is the variety Rastellino at the first location (Castel Rigone) and an unidentified variety at the second location.

PRODUCTION LOSSES AND OIL DETERIORATION DUE TO OLIVE FLY INFESTATIONS IN N.W. SARDINIA (*)

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The N.W. Sardinian olive-oil industry was studied in order to establish the relationship between *Dacus oleae* Gmelin infestations and consequent commercial losses. Three loss factors were identified, namely crop diminution due to early drop (Sept-Nov) ; reduced oil yield from attacked olives ; and oil deterioration due to increased acidity.

The analysis of samples confirmed that the degree of acidity varied directly and the oil yield indirectly with the intensity of infestation.

Totally attacked olives, picked from the plants in February showed a loss in oil yield of about 11 %, while in those picked up from the ground (dropped no more than a week previously) the yield loss was more than double.

The acidity of the totally attacked olives, picked from the plants was 2.49 % compared to 0.3 % of sound olives, while in those picked up from the ground, the acidity rose to about 4 % in the attacked ones and to 1.2 % in the sound ones.

The acidity of worm-eaten olives changes considerably, however, according to the length of time they remain on the ground ; in fact, as much as 10-15 % degrees of acidity may be registred in the olive-presses.

In 1977 the olive loss through early drop (in about 60 olive-groves) varied according to the crop abundance, the harvesting system, the olive-grove site and to the insecticide treatments.

In the treated groves, where picking from the plant is effected by means of mechanical shakers, the olives not harvested varief from 5 to 50 %. In 1976, a low-production year, 100 % was reached.

Where harvesting was carried out by shaking and simultaneously picking off the ground by hand, the unrecovered olives varied from 2 to 12 % in the treated groves (sited inland in shallow-soil areas) and from 5 to 20 % in the non-treated groves (sited in coastal, deep-soil areas).

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The commercial losses caused by *Dacus oleae* attacks can be derived from the following formula :

$$y = P_e \cdot R_1 \cdot Q_p + P_e (R_1 - R_2) \cdot Q + (P_e - P_e) \cdot R_2 \cdot Q$$

where P_e and P_1 are the prices of oil with less than 1 % acidity and more than 3 % respectively, R_1 and R_2 are the percentage oil yields of sound and attacked olives respectively, Q_p represents the olives not gathered due to *Dacus* attack and Q the oilves gathered.

TWENTY-FIVE HECTARES OF ONIONS TREATED WITH STERILE ONION FLIES (*Delia antiqua* Meig.).

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Twelve years of research went into the study of the sterile insect technique for onion fly control. Sterilization was studied by TICHELER & NOORDINK (1968) and NOORDINK (1971), histology and effects of irradiation on that level by THEUNISSEN (1976) and (1977), mass rearing by TICHELER (1972) and NOORLANDER (pers. comm.), ecology by LOOSJES (1976) and field experiments by TICHELER et al. (1974) and LOOSJES (l.c.).

The results of this endeavour indicated the feasibility of the method for control and an economic study pointed to competitiveness with chemical control with granulated insecticide.

As a consequence we were allowed to initiate the development phase aiming at application of the method on some 25 ha of commercial onions in 1978 and 1979. This required stepping-up mass production from 2 million pupae in 1973/74 to 25 million in 1977/78. As no means were available for automatization, this was achieved by rationalization ; we made things go easier and devised a strict weekly schedule of operations, using temperature in the three rearing rooms as main tool. As pupae can be stored for more than a year at 3°C, we are satisfied that we have the necessary numbers available.

In order to find a suitable area for the releases, populations were sampled last autumn on 112 onion fields, by counting on random meters of row the numbers of infested onions and by subsampling for numbers of hibernating pupae the soil around infested onions. Numbers per ha varied widely and were generally higher than we were used to, i.e. between 1 000 and 60 000. We choose an area with small and big fields, with high and low populations, at short and big distance from surrounding populations. The farmers within the area all decided to cooperate and to leave aside the insecticide treatment.

For release several methods will be compared : release of flies from aircraft taking a route over the fields or in parallel flights, release of pupae from aircraft, release of flies from a moving car and distribution of flies by a walking man. All sterile flies are labelled with different colours of dye and, as thirty colours or mixtures can be distinguished under UV light, comparisons between the release methods can be made. The effect of the releases is judged by the ratio of sterile to fertile flies caught in traps, the infestation of plants both in the area of release and outside the area, and finally by the numbers of hibernating pupae in the autumn.

The year 1979 will bu used to assess the numbers of sterile flies necessary to maintain the low level of population, hopefully achieved by the heavy releases in 1978.

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ÉTAT ACTUEL DES POSSIBILITÉS D'UTILISATION ET DES MODALITÉS D'APPLICATION DE LA LUTTE CONTRE LES GLOSSINES PAR LACHERS DE MÂLES STÉRILES.

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RESUME

Depuis une dizaine d'années nous étudions les possibilités d'application de la lutte génétique par lâchers de mâles irradiés à la mouche tsé-tsé, vecteur et hôte intermédiaire des trypanosomiases humaines et animales en Afrique.

Les problèmes technologiques auxquels se heurtait la mise oeuvre de la méthode : élevage en masse des mouches tsé-tsés ; choix du stade de l'insecte au moment de l'irradiation ; doses d'irradiation à appliquer et étude de leurs conséquences sur la longévité, la compétitivité, le comportement des mâles irradiés, etc., ont fait l'objet d'études préliminaires au laboratoire d'Entomologie de l'Institut d'Elevage et de Médecine Vétérinaire des Pays Tropicaux (I.E.M.V.T.), à Maisons-Alfort, au cours des années 1968-1972.

Des mâles de *Glossina tachinoides*, élevés à Maisons-Alfort, et expédiés après irradiation, par avion, au Tchad, ont été relachés, en 1972-1973, dans des gîtes naturels sur les fleuves Chari et Logone. Ces lâchers ont permis d'étudier le comportement de ces mâles d'élevage et ont révélé certaines contraintes quant aux modalités pratiques d'application de la méthode.

Les recherches effectuées à Maisons-Alfort et au Tchad ont abouti à la réalisation en Haute-Volta, à Bobo-Dioulasso, d'un Centre de recherche spécialisé, financé par la D.G.R.S.T. française, avec une participation de l'Office de Coopération technique de l'Allemagne Fédérale. Trois vétérinaires français, un vétérinaire allemand, trois techniciens français et une quinzaine de techniciens voltaiques y sont affectés en permanence.

Ce Centre, construit par l'I.E.M.V.T. en 1974, comprend, outre des bureaux, laboratoires, animalerie et magasin, un insectarium où sont élevées 40.000 femelles de *Glossina palpalis gambiensis* nourries quotidiennement sur lapins, dont l'effectif total comprend 400 animaux et sur cobayes (470 animaux).

Cet élevage fournit un excédent de 3.500 mâles par semaine, qui, après marquage par des tâches de peinture déposées sur le thorax et après irradiation à 11.000 rads dans un appareil comportant 4 sources au Césium 137, sont relâchés, pendant 8 mois par an, dans des galeries forestières situées à 70 km à l'Ouest de Bobo-Dioulasso, abritant des populations sauvages de *G. palpalis gambiensis*, espèce dominante et de *G. tachinoides*, plus faiblement représentée.

Le premier objectif était d'évaluer l'efficacité de la méthode en appréciant, par des observations régulières, la capacité de dispersion et la longévité des mâles irradiés et par l'étude de la dynamique des populations (densité, sex ratio, rapport des mâles stériles aux mâles sauvages, etc.) et le contrôle de la fertilité des femelles sauvages, leur impact sur la population naturelle.

Les études de comportement ont montré une excellente adaptation des mâles irradiés sur le terrain. Les dernières observations révèlent une diminution considérable de la population sauvage de *G.p. gambiensis*, de *G.tachinoides* s'est maintenue à son niveau antérieur.

Les lâchers seront poursuivis et étendus en 1978-1979, pour étudier les différents paramètres permettant de rendre la méthode compétitive par rapport aux méthodes classiques de lutte.

La seule limite que l'on voit à la méthode, en dehors de son organisation stratégique pour une action d'ampleur, réside dans l'incertitude de pouvoir l'appliquer à toutes les espèces de glossines, notamment à celles qui vivent largement dispersées dans les savanes soudaniennes et sont dès lors peu accessibles aux mâles stériles, dont l'efficacité ne peut se manifester que sur des populations isolées et de faible densité.

TESTS FOR MATING COMPETITIVENESS IN THE FIELD

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The ability to compete for mates in the field is the essential characteristic required of males to be used for genetic control. It is scarcely worthwhile to carry out laboratory quality control tests unless the results are correlated with mating competitiveness in the field. Field tests of competitiveness require collection of data on the relative numbers of released and wild males and of the relative numbers of matings by each type of male. The presence in the experimental area of females inseminated before the males were released or females which immigrated after insemination can confuse the results, but such confusion can be avoided by release into the experimental area of marked virgin females, recapture of the females after mating and evaluation of the level of sterility in their eggs. Experiments in Asia, America and Africa with various genetically altered forms of *Aedes aegypti* will be reviewed and the applicability of this method to other insect pests will be considered.

TRANSLOCATIONS AND INVERSIONS INDUCED IN *Hylemya antiqua* (MEIGEN) WITH X-RAYS OR FAST NEUTRONS. FERTILITY DATA, CYTOLOGY AND BREAKPOINT DISTRIBUTION.

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In the context of genetic control of the onion fly, adult flies were exposed to X-rays or fast neutrons to induce chromosomal rearrangements which possess "semi"-sterility.

A dose-response curve for both types of radiation administered to adult males is given. After a screening for inherited "semi"-sterility during two generations followed by cytological examination, 55 translocations and 5 pericentric inversions were isolated. The level of sterility of the isolated rearrangements varied from 30 % to 70 %. The observed breakpoint distribution over the chromosomes, or their arms, was compared with the expected distribution on the basis of chromosome (arm) length. Chromosomes 2 and 6 appeared to be somewhat more involved in the rearrangements than the other chromosomes. Fast neutrons given at low doses '.1 - .2 krad) yielded a good output of rearrangements as compared to X-rays. Several translocations were sibcrossed to isolate homozygous stocks with a limited success.

FIELD HOUSEFLY POPULATIONS POLYMORPHIC FOR SEX DETERMING MECHANISMS.

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Various sex determining mechanisms have been described in the housefly, based either on sex chromosomes or on male and/or female autosomal factors. Scattered evidence seemed to suggest that the XX, XY mechanism was the commonest one and that only it it was present in Europe and in the Northern United States and Canada. Mechanisms based on autosomal factors, both sexes having twoX chromosomes seemed limited to populations from Southern USA and some other widely separated (warm) localities.

These different mechanisms have been successfully submitted to sophisticated laboratory manipulations, showing that they can coexist and be variously combined. However no evidence of mixed field populations had been reported until very recently : field populations seemed to fall into either one or the other of two quite distinct categories.

During the last two years, my colleague DrP.G. RUBINI has found repeated evidence of mixed housefly populations from various european localities, and of a quite wide distribution of XX males.

Coexistence of different sex determing mechanisms and consequent excess of males seem now quite widespread in Europe. Similarly mixed populations have been recently mentioned in the U.S.A. In view of the present situation, it seems rather surprising that it may have excaped attention before.

If this particular form of polymorphism is a new phenomenon, its wide distribution in Europe would imply a very high spreading power and so high selective advantage.

Some of the Rubini's populations have been tested for insecticide resistance. All turned out highly resistant to both chlorinated and OP insecticides. Some of these populations came from very remote mountain places, very unlikely sites for direct insecticide selective pressure.

LARVAL CULTURE MEDIUM CONDITIONING AND MATING CHOICE IN THE HOUSEFLY

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Two years ago, my colleague Dr. P.G. RUBINI has given to this working group evidence that housefly males are preferentially attracted by females raised on their own culture, independently from the strain to which they belong.

The early data regarded flies from long established laboratory strains raised on standard medium, observed on Y shaped olphactometers.

The original findings have been fully confirmed by comparing wild-type flies of recent colonization with flies from a wild-type standard strain (WHO) raised either on standard medium or on horse dungs. Attraction and mating choice have been tested both with the Y shape olphactometer and by counting successfull matings of males having two choices in mating cages.

When flies of different strains are raised on the same medium, matings are largely more frequent between flies of the same wild-type strain ; when are raised on different media, the choice is strongly directed by the medium on which flies had been raised.

It is not yet clear if the intra-strain mating preference mentioned above is directed by strain properties or rather by the fact that both sexes came from the same larval jar.

These findings indicate that at least in laboratory house flies mating preferences are strongly canalized during larval life, a fact which may badly affect the sexual competitiveness in the field of otherwise quite carefully rised laboratory males.

GENETIC POLYMORPHISM FOR 6-PHOSPHOGLYCONATE DEHYDROGENASE (6-PGD) IN THE HOUSEFLY.

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Genetic polymorphism for enzyme systems provide a highly reliable tool for population genetics studies and for research works requiring the use of genetic markers.

My colleagues Dr. G. GASPERI and Dr. A. MALACRIDA have found different forms of enzyme 6-PGD both in various laboratory strains and in wild-type strains of recent colonization.

Two forms of this enzyme have been isolated from strain WHO/IN/M.d./1. These forms differ for electrophoretic mobility and staining intensity. Their phenotypes can be described as "fast-weak" and "slow-thick" ; the heterozygous phenotype is asymmetrical, showing both bands plus an intermediate one ; the intermediate band is typical of the heterozygous flies and does not appear from in vitro mixtures.

The different staining intensities of the two forms may result from differences either in stability or in activity.

Genetical tests show clearly that two alleles are involved : $\underline{Pgd}^{A}$, causing the fast-weak phenotype and $\underline{Pgd}^{B}$, causing the slow-thick one.

The locus Pgd has been assigned to linkage group III, which carries also the genes kdr (knockdown DDT resistance) and M (maleness) ; it is located some 30 crossing-over units apart from the marker bwb. These properties seem quite valuable for genetic manipulation programs.

The long established strain bwb dv kdr has been found homozygous for a third form of the same gene ($\underline{Pgd}^{C}$), which causes a thick-fast phenotype ; in the heterozygous conditions it masks completely $\underline{Pgd}^{A}$ and gives a three-band symmetrical picture with $\underline{Pgd}^{B}$.

$\underline{Pgd}^{A}$ and $\underline{Pgd}^{B}$ have been found in a series of laboratory and wild-type strains.

So far $\underline{Pgd}^{A}$ (fast-weak) has been found in the homomorphous condition only in intentionally established strains.

ACTIVITY OF MIXED-FUNCTION OXIDASES IN *Ceratitis capitata* WIEDEMANN.

Pietro SCOPPA (+) & Raffaele CAVALLORO (¥)

ABSTRACT

Considerable researches on integrated pest management are in progress and as a consequence and integration of all methods as well as alternative strategies of pest control, have received increased attention.

The sterilizing treatment of insect pests in the prospect of the application of genetic control by sterile-insect technique, can influence, among other important quality traits, the behaviour of the insect.

In view of possible effects of ioninzing radiations on the sensitivity of insects to pesticides, three types of drug oxidation reactions were studied in enzyme preparations from abdomens of 4-day-old *Ceratitis capitata* adults, i.e. aminopyrine N-demethylation, p-nitroanisole O-demethylation and aniline hydroxylation.

Enzyme assays, carried out using well established methods for studying "in vitro" the metabolism of foreign compounds in insects, indicated the microsomal localization of mixed function oxidases and suggested that the species *Ceratitis capitata* is a slow metabolizer of these prototype drug substrates.

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PRELIMINARY OBSERVATIONS ABOUT THE TEMPORARY RESISTENCE OF FRUITS OF SOME SPECIES OF CITRUS FRUIT TO THE ATTACKS OF *Ceratitis capitata* Wied.

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Preliminary observations about the temporary resistance to the attacks of *Ceratitis* of some varieties of citrus fruit during the period preceding the physiological ripening of the fruit, have been carried out.

After having determined the colouring index of epicarp its thickness and the glands number per cm^2, which varies considerably among the different tested species and cultivars, the insect eggs have been treated with the essential oil extracted by pressure.

It has resulted (Tab. 1) a lethal oil effect on *Ceratitis* eggs ; in fact the hatcking referred to the control, has changed from a minimum of 19 % in cv. Tarocco Orange to a maximum of 49 % in cv. Moro Orange.

The glands number and the epi-mesocarp thickness vary considerably during the maturative development of fruits, for instance, in the cv. W. navel the glands number per cm^2 passes from 202 to 68 with the variation of epi-mesocarp colouring index from 3.7 to 9.5 and of ripening index from 3.5 to 6.6, while the epi-mesocarp thickness passes from 8.5 to 6.2 mm.

The resistance of citrus fruit to *Ceratitis* infestation, expressed with the maximum values of essential oil glands per cm^2 and of epi-mesocarp thickness, is particularly important because it coincides with the period of greatest density of flies in the field (October-November).

From the above-mentioned it seems extremely interesting to continue the research in this direction with the purpose to locate, in the ambit of the species spread in our environment, those cultivars that, for their morphological features give the greatest guarantees of resistance to the attacks of the Tripetides.

Tab. 1 - Preliminary data about the mortality of C. capitata Wied. eggs in essential oils and about the morphological variations of epi-mesocarp of some species of citrus fruit during the period of fruits ripening.

Citrus fruit cultivars	% of hatched eggs in essential oils	FRUITS									Ripening period
		GREEN				HALF-RIPE				RIPE	
		Epi-mesocarp			Endocarp	Epi-mesocarp			Endocarp	Endocarp	
		Thickness	Colouring	cm^2 glands number	Ripening index	Thickness	Colouring	cm^2 glands number	Ripening index	Ripening index	
Orange:											
W. navel	30	8.5	3.7	202	3.5	6.2	9.5	68	6.6	13	December
Moro	49	6.5	4.8	183	5.0	3.4	8.0	56	5.2	10	January
Tarocco	19	7.0	5.5	95	5.2	5.2	9.5	76	5.4	9.6	February
Valencia	27	4.3	4.7	110	3.5	3.5	5.5	83	3.5	14.3	April - May
Clementine:											
Common Clementine	43	2.8	4.2	119	10,55	2.3	10.5	59	11.9	13	November
Mandarins: Mandarine:											
Avana	65	2.4	4.0	119	4.5	3.6	5.5	75	6.9	9.5	December-January
Satsuma	42	5.0	6.5	135	6.0	4.4	10.0	56	9.9	11	November-December
Grape-fruit:											
Frost Marsh	22	15.2	4.7	130	4.3	11.8	6.5	48	3.8	6	March
Control in H_2O	100										

CROSSING EXPERIMENTS WITH DIFFERENT POPULATIONS OF *Rhagoletis cerasi* L. IN GERMANY (*)

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The populations, collected from infested fruits, have usually been crossed in single pairs. The copulation frequencies of the pairs were observed at equal time spans of one or a half hour for 7 hours per day during the first week after hatching. It could be shown that the females from north of a thought border line yield no offspring crossed with males from the south of this line. It runs somewhere between Frankfurt/Mainz and Franconia. However, the date do not allow to speak of only two (plasma-) incompatible races, because sterility was also observed within the "northern" or "southern" groups of populations.

It should further be noted that also significant differences of the copulation frequencies could be stated (but without any connection with the fertility of a pair). That is astonishing, because in field observation the males mediated the impression that they are guided to the females only by optical stimuli. It is sure that the mating behaviour of captivated flies and such in the field differs much. But the observation may be a hint that we -in intending to apply any genetic control technique- should not loose sight of the mating readiness of individuals between the populations.

(*) Work was supported by the Deutsche Forschungsgemeinschaft.

TWO YEARS OF BIOECOLOGICAL OBSERVATIONS ON *Rhagoletis cerasi* IN DIFFERENT ITALIAN AREAS.

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After a first report on Cherry fruit fly (Wadenswil, september 1975) concerning only the South of Italy (Campania) four Italian Institutes of Entomology -in the framework of C.N.R. Projects (National Council of Researches)- carried out in the last two years (1976 and 1977) joint researches on flies trapping and fruit infestation in different areas.

Beside two important areas, Naples and surroundings (20 % of Italian production) and Bari (7 %) two others of extreme latitude, Piacenza in the North and Catania in the insular South (Sicily) were investigated. The number of flies was weekly observed by yellow coated traps (O.I.L.B. "Prokobol") and the larval infestation was followed by laboratory examination of fruit samples.

The catches of flies in 1976 started at beginning of May (a little delayed than usual) in some fields near Naples and at the half of this month in others, in Sicily and Apulia. They continued in many cases till the end of June. In Sicily and in the Emilia areas, northern, the first flies appeared later.

The number of flies caught was generally very low in some fields of Apulia with a corresponding infestation at harvest time varying around 2 - 10 %, and low in Emilia, with a 25 - 35 % of wasted fruit. In some fields of Campania the number of flies was very high and the infestation often more than 90 - 95 %, caused by many larvae in the same fruit.

In fields of Sicily to an intermediate degree of catches corresponded almost complete product wastage (more than 95 %) while in others of Campania the same degree of catches or a higher one gave low infestation, not exceeding in some cultivars 25 %.

The local climatic conditions, the cultivars, plant phenelogy and obviously the presence of fruit fly population influenced by them, constitute the principal parameters conditioning the intensity of fruit infestation.

(*) The authors are responsible of C.N.R. Oper. Units-Fruit Fly project coordinated by Prof. G. FIORI, Università di Perugia (Italia)

The researches carried out jointly in so different parts of Italy contribute to a better understanding of local situations and of their meaning in timing spray interventions or other control techniques.

HOST INFLUENCE ON THE DIAPAUSE OF *Rhagoletis cerasi* LINNAEUS 1758 (*)

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The main hosts of *R. cerasi* are *Prunus avium* and *Linocera* ssp. The latter one bears fruit later in the season than the sweet cherries do. An obligate diapause during the pupal stage effects the phenological adaption of *R. cerasi* to each host. Indeed the diapause of *R. cerasi* out of *Lonicera* fruits lasts at least three weeks longer than that of animals out of sweet cherries does.

Different temperature treatments have been employed (Table 1) to study the diapause character of both populations. The populations were :

1) Prunus population from a typical prunus site (=Hetzles),
2) Lonicera from an exclusive lonicera site Schäftlarn 250 km away,
3) Lonicera from Schäftlarn released on artificially under Prunus-trees plantes Lonicera bushes at Hetzles.

The mean hatching rates and the time needed for the post-diapause development is shown by the figure. Both criteria displayed a medium character of the Lonicera-population from Hetzles lying in between those of the genuine Prunus resp. Lonicera populations. They let appear the population Lonicera/Hetzles as a mixture of both populations. However, no correlation could be proved between the hatching rates or diapause development of the populations Prunus/Hetzles and Lonicera/Hetzles or Lonicera/Hetzles and Lonicera/Schäftlarn. It must be concluded that the food influenced the diapause. It is assumed that the released population Lonicera/Schäftlarn in fact vanished and that the population Lonicera/Hetzles consisted of individuals of the endemic population Prunus/Hetzles. These, however, grown up in Lonicera-fruits adopted a more intensive diapause under the changed nutritional conditions for the larvae.

(*) Work was supported by the German Bundesministerium für Forschung und Technologie.

Tab. 1 Temperature Treatment of Rhagoletis cerasi pupae.

Cold Exposition time (d)	Exposition temperature (d) °C 5	2	0	2	5
80	10	15	30	15	10
80	15	20	10	20	15
80	10	15	55	-	-
80	10	70	-	-	-
80	15	65	-	-	-
100	10	15	50	15	10
100	15	20	30	20	15
100	10	15	70	15	10
100	15	20	50	20	15
100	10	15	55	-	-
100	15	20	45	-	-
120	10	15	70	15	10
120	15	20	15	20	15
120	10	15	95	-	-
120	15	20	85	-	-
120	10	110	-	-	-
120	15	105	-	-	-

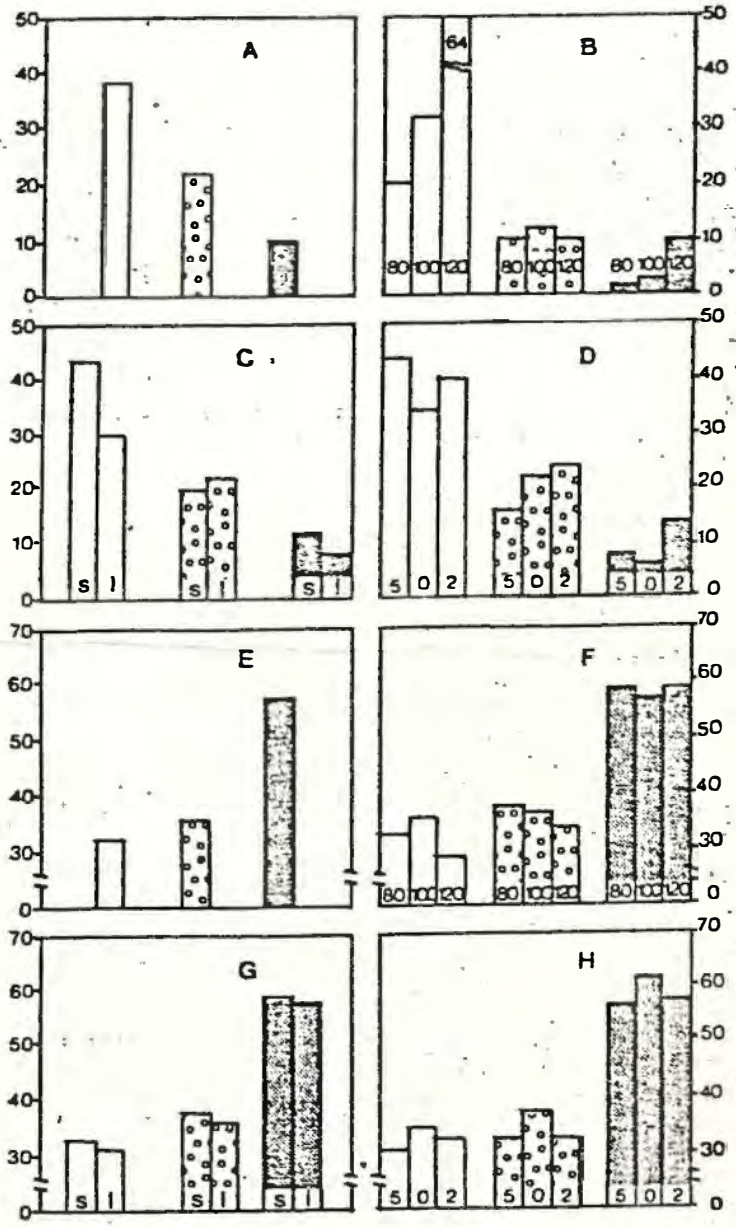

Fig.:
Effects of temperature treatment on pupae of R. cerasi:
A - D: Hatching rates (%); E - H: Postdiapause development (d)
A and E Populations: Prunus/Hetzles [—], Lonicera/Hetzles [- -], Lonicera/Schäftlarn [▨]; B and F: cold exposition (d); C and G: fast (s) and slow (l) temperature decrease; D and H: last temperature during diapause.

INVESTIGATIONS ON THE DISTRIBUTION OF THE UNIDIRECTIONAL INCOMPATIBILITY OF THE EUROPEAN CHERRY FRUIT FLY IN AUSTRIA.

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Because of the discovery of unidirectional incompatibility in the European cherry fruit fly, *Rhagoletis cerasi* L., and of the fact that the border between the two naturally incompatible races lies in Lower Austria, it was desirable to clarify as exactly and completely as possible the dispersion of the two races in Austria.

Within the last four years it was possible to make extensive pertinent investigations.

As could be shown, the course of the border between both races does not correspond according to present knowledge to any topographical, climatic or other ecological parameter. It was discovered that the border is not a sharp delineation but rather a more or less broad border area which is populated by both races in differing proportions.

It can be assumed that there is a so-called "Mixed-zone", within which nearly every ration between both races is possible.

More details will be given during the meeting.