IOBC/WPRS

Working Group

"Fruit Flies of Economic Importance"

OILB/SROP

Groupe de travail

"Mouches des Fruits d' Importance Economique"

and IICT

International Open Meeting

at/à

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Edited by J. Piedade-Guerreiro

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FOREWORD

The enormous spreading and the increasing losses due to fruit flies of economic importance all over the world and particullarly in the Mediterranean Region, lead the International Organization for Biological Control / West Paleartic Regional Section to mobilize it's means for the implementation of actions that enable the control of such pests.

In this context the IOBC/WPRS Working Group "Fruit Flies of Economic Importance" organized the "International Open Meeting", under the high patronages of the Instituto de Investigação Científica Tropical (IICT) and the mentioned International Organization.

This meetings usually take place every four years and have for porpose the:

- Establishment of contacts and exchange of knowledge between specialists in this area of science and also to enhance efforts for a wider cooperation between the differents Subgroups.

- Evaluation of the progress made in the improvement of the control tecnhiques and identification of new areas for future programs involving laboratories in different countries, aiming for the setting of an effective protection without negative consequences not only for man but also for the environnment.

We hope that the friendly spirit and enthusiasm that animated this "International Open Meeting" in wich participated more than eighty cientist of sixteen contries, that presented forty communications may be materialized in concrete and effective control of one of the great nuisances of agriculture: Fruit flies.

Finally we must not forget the cooperation and support not only of the IOBC/WPRS namely of it's President Prof. R. Cavailoro and the Secretary General Dr. S. Poitout, but also of the IICT Presidence especially of the Prof. A. Réffega, whose confidence, support and incentive greatly contribuited to the sucess of this Meeting.

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S.I.T. Sterile Insect Technique	Piedade-Guerreiro	J. Hendrischs P. Liedo A. Wakid
Integrated Control Programe	Kurt Russ	E. Boller A. Economopoulos

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Opening session

Chairman: J. A. CRUZ E SILVA
Secretary: J. PIEDADE-GUERREIRO

Welcome adress
J. A. Cruz e Silva
Presidente do Instituto de Investigação Científica Tropical

Constitui para mim, como Presidente do Instituto de Investigação Científica Tropical, anfitrião e promotor deste Encontro, motivo de grande honra e muita satisfação poder dirigir estas breves palavras a tão ilustres congressistas aquando da abertura solene deste "International Open Meeting".

Como julgo ser do conhecimento de V. Exas, é o primeiro a ter lugar em Portugal ligado ao Grupo de Trabalho "Mouches des Fruits d'Importance Economique" da Organização Internacional de Luta Biológica e Integrada - Secção Regional Oeste Paleárctica, o que, obviamente, constitui para este Instituto e para este País motivo de fundamentado e saudável orgulho.

Na verdade, a concretização desta Reunião muito dignifica a Comunidade Científica Nacional e Internacional e particularmente os especialistas em Luta Biológica, a qual se vem afirmando cada vez mais como um instrumento poderoso na luta contra pragas das culturas.

Naturalmente que não me quero alongar nestas minhas breves palavras.

Todavia não posso deixar de tecer algumas considerações, obviamente sucintas, acerca das moscas da fruta de importância económica.

Estas são fundamentalmente consideradas como uma das grandes pragas das fruteiras a nível mundial e a sua acção reflecte-se, de modo muito nefasto, na economia de muitos países, nomeadamente em Portugal Continental e Regiões Insulares onde, na Região Autónoma da Madeira, por exemplo, a espécie *Ceratitis capitata* tem vindo a recrudescer nos últimos tempos provocando anualmente prejuízos superiores a meio milhão de contos.

Os temas que vão ser debatidos neste Encontro Internacional têm por fim fazer o ponto da situação dos conhecimentos actuais e encontrar novas orientações no combate às moscas da fruta de importância económica.

Desta forma, pretende-se estabelecer, não somente estratégias convenientes para o seu combate, mas também definir novas áreas de investigação ligadas ao campo da luta biológica e da protecção integrada.

Sem procurar entrar em pormenores, direi que podemos considerar que foi a partir da década de cinquenta que na Europa a luta contra insectos nocivos se começou a orientar no sentido da luta biológica e integrada, principalmente com o incentivo dado pela Organização Internacional de Luta Biológica e Integrada, fundada em 1956, tendo o Instituto a que tenho a honra de presidir sido um dos seus Membros Fundadores.

Em 1971 foi constituída a Secção Regional Oeste Paleárctica (SROP) tendo um dos seus investigadores, Dr. Armando Castel-Branco, sido nomeado presidente da Comissão de Gestão na altura em que ocupava o cargo de Vice-Presidente da OILB.

Foi com o incentivo dado por esta Instituição que o IICT empreendeu na década de cinquenta as primeiras acções de luta biológica realizadas no continente africano, numa altura em que grande parte dos especialistas mantinha uma atitude bastante passiva ou mesmo de inaceitável cepticismo em relação a estas matérias.

Dos muito programas de Luta Biológica implementados no referido continente, gostariamos de salientar apenas dois deles: na Ilha do Príncipe a luta contra a cochonilha dos coqueiros *Aspidiotus destructor* que, em 1955, ameaçava destruí-los e que pela introdução do coccinelídeo *Cryptognatha nodiceps*, a partir de 1956 ficaram totalmente recuperados; e na Ilha de São Tomé, onde foi introduzido com grande sucesso o predador da icéria (*Icerya purchasi*) *Novius cardinalis*.

Estas campanhas de luta biológica, as primeiras realizadas no continente africano tal como disse, revelam bem a necessidade de se abandonarem os processos empíricos de aplicação de insecticidas e de se enveredar pelo estudo das biocenoses relativamente aos insectos nocivos e dos seus parasitas e predadores.

É neste contexto que a Secção Regional Oeste Paleárctica tem desenvolvido uma acção importante de coordenação no campo da investigação e das técnicas de combate, através de diferentes Grupos de Trabalho os quais têm contribuído, de forma muito notória, para o desenvolvimento de uma agricultura sustentada, capaz de preservar a nossa qualidade de vida contribuindo para uma gestão racional dos precários ecossistemas naturais e antrópicos.

Foi nesta óptica que se constituiu, em 1978, o Grupo de Trabalho "Mouches des Fruits d'Importance Economique" que teve como primeiro Convenor o Dr. E. Boller da Suiça, sucedendo-lhe o Prof. Cavalloro e o Prof. Delrio, ambos de Itália, com grande prazer meu todos aqui presentes e a quem dirijo uma palavra especial de felicitações, cabendo agora e pela primeira vez a responsabilidade do Grupo a um investigador do Instituto de Investigação Científica Tropical, Doutor Piedade-Guerreiro, o que muito me apraz registar.

Estou certo de que todos nós estamos cientes da grande importância desta Reunião respeitante a uma área do conhecimento científico que embora restrita é no entanto de enorme alcance do ponto de vista económico e cuja orientação será decisiva para a protecção das fruteiras dentro da área de influência da Secção Regional Oeste Paleárctica.

Não tenho dúvidas de que este "International Open Meeting" será enriquecedor em termos científicos e humanos, tendo em atenção a elevada e bem conhecida especialização dos seus participantes pertencentes a dezasseis países que passo a referir por ordem alfabética: Alemanha, Argélia, Argentina, Austria, Egipto, Espanha, França, Grécia, Inglaterra, Israel, Itália, Marrocos, México, Portugal, Suiça e Tunísia.

Termino minhas Senhoras e meus Senhores desejando a esta vasta plêiade de Congressistas cuja presença muito nos honra um trabalho profícuo e uma boa estada entre nós e deixando uma palavrea de estímulo e de reconhecimento a todos aqueles que deram o melhor do seu esforço na preparação deste "International Open Meeting" que não tenho dúvidas constituirá um enorme êxito científico.

Adress de bienvenue et evolution du Groupe

Raffaele Cavalloro
Président OILB / SROP

Il m'est très agréable d'exprimer d'abord ma plus grande appréciation au Prof. J. A. Cruz e Silva pour sa cordiale bienvenue et d'adresser un remerciement chaleureux aux Proff. V. Garcia, P. Amaro et K. Russ pour avoir bien voulu être avec nous à cette réunion plénière du Groupe de travail OILB/SROP "Mouches des fruits d'importance économique".

A tous les éminents participants et aux nombreux collègues et amis, que j'ai le grand plaisir de rencontrer et de voir parmi nous, la bienvenue la plus vive de ma part et aussi au nom des membres du Conseil de l'OILB/SROP.

Nous sommes très reconnaissants à nos collègues et hôtes portugais, notamment au Prof. J. Piedade-Guerreiro, qui ont voulu nous inviter à siéger ici, à Lisbonne, laquelle nous accueille avec son charme de toujours.

Le Groupe de travail a 15 années de vie: il prend naissance le 15 mai 1978 du fusionnement des Groupes de travail préexistants tels que ceux concernant la *Ceratitis capitata* Wied., la *Rhagoletis cerasi* L., les ravageurs de l'olivier et celui sur les méthodes génétiques dans la lutte contre les ennemis des cultures.

Le premier noyau de ce nouveau Groupe était constitué par 13 spécialistes de 7 Pays. Sous la direction de Mr. E. F. Boller (CH), 5 sous-groupes animés par des responsables avaient le but prioritaire de mettre en place un programme concret de lutte intégrée surtout contre *Ceratitis capitata*, *Dacus oleae* Gmel., *Rhagoletis cerasi*, en demarrant avec des recherches et des actions à bref et à long terme.

Le Groupe a évolué si rapidement que déjà avec la gestion suivante, sous la direction de Mr. R. Cavalloro (I), il comprenait 79 experts participants, de 25 Pays plus 2 organisations internationales, chargés d'activités en collaboration touchant d'importants sujets tels que l'écologie, la génétique des populations, le comportement, la physiologie, la nutrition, les problèmes des symbiontes, les lignes cellulaires "in vitro", les relations hôte-parasite, la taxonomie, la lutte biologique les microorganismes, les espèces entomophages, l'élevage en masse, le contrôle de qualité, le recours à modèles mathématiques, les méthodes biotecniques, le piégeage et les attractants, les phéromones et d'autres médiateurs chimiques, la technique des insectes stériles, la lutte chimique et le recours à substances nouvelles, les stratégies de lutte intégrée, la réalisation de programmes de IPM, etc.

Successivement, l'augmentation du nombre des experts intéressés, s'élevant à 256 dont 68% au déhors des Pays de la SROP, et l'accroîssement d'intérêt pour les activités du Groupe qui allaient au-delà de l'engagement régional, ont poussé l'OILB-Globale à créer un autre Groupe de travail parallèle et similaire, mais orienté plutôt vers sujets plus généraux.

Cette réalisation s'est avérée sous la direction suivante de Mr. G. Delrio (I) qui a été donc appelé à une restructuration du Groupe SROP, avant de le confier à Mr. J. Piedade-Guerreiro (P) qui en assure à présent la direction et qui le gère avec art et savoir suivant lignes d'action régionales et complémentaires au Groupe Globale et en bonne entente avec son actuel responsable Mr. P. Liedo (MEX).

En une dizaine d'années, donc, les remarquables progrés du Groupe, très actif et bien motivé, l'ont amené à une véritable prolifération et à une très positive 'crise de croîssance'.

La témoignent aussi les importants progrès, les nombreuses réalisations et publications, dont on peut rappeler 12 réunions d'experts et responsables en Allemagne, Espagne, Grèce, Italie et Portugal, 1 cours de formation sur le contrôle de qualité, 3 symposium internationaux en collaboration avec la Comission des Communautès Européennes.

Cette réunion plénière fera donc le point et montrera les futures actions d'un Groupe OILB/SROP qu'on peut appeler nouveau mais qui a un héritage de grande envergure et qui est certainement destiné à jouer un rôle fort important dans le domaine de la lutte intégrée.

Il nous attend un travail engagé, mais il est certain, de grande utilité et riche en satisfactions.

Mes meilleurs voeux de succès à tous les participants.

Welcome adress
Vasco Garcia
Member of the European Parliament

Os membros da Comissão de Agricultura e Pescas, de que sou titular desde que Portugal aderiu às Comunidades Europeias, estão habituados a tarefas difíceis, às vezes quase impossíveis.

Mas algumas missões são muito reconfortantes e desempenham-se com facilidade natural de quem regressa ao meio dos seus pares.

Participar neste Encontro Internacional sobre "Moscas das Frutas de Importância Económica" é regressar às origens. E não podem imaginar o sabor que tem, talvez só comparável ao gosto que a mosca do Mediterrâneo sente pelos frutos das argânias, lá no Atlas marroquino...

Aliás, a floresta de *Argania sideroxylon* tem algo que ver comigo, porque é considerada uma relíquia da flora Macaronésica, tal como algumas zonas de vegetação das nossas ilhas dos Açores e da Madeira. E ainda porque tive de a estudar, ao orientar e arguir a tese do Presidente deste "Meeting" Doutor João Piedade-Guerreiro, a quem aproveito para felicitar com Amizade, mas também com justiça, pela organização impecável e por ter conseguido em tempos tão difíceis para a Europa, reunir tantos participantes e de tão grande qualidade.

Isto prova que a nossa Europa está viva e que com ela caminham países nossos vizinhos e amigos, seja na definição das grandes políticas ou na procura de soluções para problemas específicos e de carácter científico, como é o caso das moscas da fruta.

Numa altura em que a reforma da Política Agrícola Comum dá uma importância particular aos aspectos ambientais, canalizando para tais fins meios financeiros que ninguém imaginava há uns anos atrás, é de recordarmos que estavam certos todos aqueles que sempre defenderam os métodos biológicos e bio-técnicos de controlo de pragas. Talvez se os políticos tivessem escutado mais os defensores da luta biológica e da luta integrada dispusessemos hoje de meios eficazes, não poluentes, rentáveis e competitivos para reduzir os impactos negativos das pragas e doenças das plantas e animais.

Mesmo a CE tem sido um pouco falsa neste aspecto, pois é sempre um palco fácil para pressões de "lobbies". O exemplo máximo é o Parlamento Europeu, onde em 8 anos pouco ou nada ouvi falar de luta biológica, dado o seu pouco interesse industrial.

E no entanto, já KARPATI em 1983 estimava em 25 milhões de dólares os prejuízos anuais devidos somente à "med-fly" nos países e regiões infestados.

O México estimou em cerca de 500 milhões de dólares os prejuízos da fixação da *Ceratitis capitata.* E a criação da NAFTA - North American Free Trade Agreement só veio dar mais realismo ao aparecimento destes problemas, do mesmo modo que o Mercado Único Europeu de 93 vem provar que no domínio fito-sanitário é cada vez mais importante a colaboração entre especialistas dos Estados-membros. Mais; é necessário estender essa cooperação aos países candidatos à adesão europeia, tornada agora mais viável e atraente pelo acordo que o Tribunal Constitucional alemão deu ao Tratado da União Europeia.

Na mesma linha estão os países terceiros, para quem a União Europeia se afigura, em muitos casos, o grande parceiro do futuro. Iniciativas como esta são essenciais para os nossos investigadores, para o alargamento da Ciência europeia.

Nós Portugueses, detentores duma velha cultura e membros de direito da Nova Europa, só temos que nos congratular e desejar sucesso - muito sucesso! - a este Encontro.

Prestamos assim homenagem a vultos como PEREIRA COUTINHO, que em 1898 fez a primeira referência à *Ceratitis capitata* em Portugal, assim como a VERÍSSIMO D'ALMEIDA, a Mac LEAY, a NEWMAN, a BALACHOWSKY, a CASTEL-BRANCO, cientistas das gerações passadas que tanto tempo dedicaram aos humildes dípteros de que nos ocupamos.

E olhando o passado rico que nos legaram, encaremos com confiança as novas gerações que lhes sucederam e sucederão. Porque se houver uma Europa eterna, ela será sempre a da Ciência e do Espírito que a vossa presença aqui em Portugal tão bem simboliza.

Welcome adress

P. Amaro
Président de la Société Portugaise de Phytiatrie et Phytopharmacie

1 - Permettez-moi, avant tout, de remercier le Dr. Piedade-Guerreiro pour sa gentillesse en m'invitant à participer, en tant que Président de la Société Portugaise de Phytiatrie et Phytopharmacie, à la session inaugurale de cette Réunion du Groupe de Travail des Mouches des Fruits d'Importance Économique que l'Organisation Internationale de Lutte Biologique et Intégrée a décidé de réaliser à Lisbonne.

2 - La Société Portugaise de Phytiatrie et Phytopharmacie développe ses activités depuis 32 ans, à travers le fonctionnement de groupes de travail et de l'organisation de réunions.

Outre la réalisation du 1er Congrès Portugais de Phytiatrie et Phytopharmacie, à Lisbonne, en Décembre 1980, qui a proportionné, à plus de 500 participants, le bilan des connaissances disponibles, alors au Portugal, et une évaluation des structures de la recherche, enseignement et extension dans le domaine de la Protection des Plantes, on distingue les initiatives en trois domaines:

a) aspects toxicologiques des pesticides (ex: classification toxicologique, délais de sécurité, précautions toxicologiques) et méthodes d'essai biologique d'insecticides, acaricides et fongicides, spécialement dans les années 60 et 70;

b) herbologie, la normalisation des méthodes d'essai des herbicides, et la réalisation de quatre Symposiums d'Herbologie (1971, 1976, 1980 et 1986) et de deux Réunions Internationales sur Herbologie Méditerranéenne, ces dernières en collaboration avec le Conseil Européen d'Herbologie (EWRC), en 1966, et la Société Européenne d'Herbologie (EWRS), en 1984.

c) protection intégrée. à travers la réalisation de quelques réunions sur Auxiliaires et Produits Phytopharmaceutiques (Mai 1991), Protection Intégrée en Pommier et Poirier (Déc. 1991), Solarisation du Sol (Juin 1992), Congrès sur les Agrumes, où sont inclus les Symposiums sur Production d'Agrumes et sur Protection Intégrée d'Agrumes (Jan. 1993) et Production Intégrée du Pommier et Poirier (Mai 1993); la prochaine réalisation de la 1ère Réunion de Lutte Biologique (17, 18 et 19 Jan. 1994) et un Workshop sur l'Enseignement de la Protection Intégrée (Avril 1994).

3 - L'activité méritoire du Groupe de Travail de l'OILB/SROP concernant les Mouches des Fruits est bien connue, ayant beaucoup contribué pour le progrès de la recherche et des connaissances sur le sujet.

Au Portugal, sont particulièrement importants les dommages causés, à divers hôtes, par *Ceratitis capitata*, et aussi par la mouche de l'olive, *Dacus oleae* et sur une plus petit échelle par la mouche de la cerise, *Rhagoletis cerasi*, pour ne pas mentionner d'autres espèces.

Sont attendues, avec la plus grande expectative, les nouveautés sur la lutte contre la mouche des fruits, *C. capitata*, qui continue à provoquer, nommément au pêcher, aux agrumes et aux fruits à pépins (dans ces derniers avec moins d'importance), des difficultés aux systèmes de protection intégrée, étant donné l'agressivité pour les auxiliaires des insecticides utilisés, reduite à travers l'utilisation d'appâts, méthode peu généralisée au Portugal.

Pour terminer et à propos de ce ravageur d'une si grande importance économique à l'île de Madeira, où la liste des hôtes est si vaste, nous félicitons le Dr. Piedade-Guerreiro pour l'intense activité développée, en collaboration avec les autorités de l'île, pour que l'on puisse mettre en pratique, prochainement, souhaitons-le, un ambitieux projet de lutte autocide pour combattre *C. capitata* à l'île de Madeira, financé partiellement par la Communauté Européenne.

Tableau 1 - Iniciatives de la Société Portugaise de Phytiatrie et Phytopharmacie

Année	Mois	Lieu	Thème	Collaboration
1966	Juin	Oeiras	1er Symposium Méditerranéen d'Herbologie	EWRC
1971	Déc.	Oeiras	1er Symposium National d'Herbologie	
1976	Déc.	Oeiras	2ème Symposium National d'Herbologie	
1980	Déc.	Lisbonne	3ème Symposium National d'Herbologie	
1980	Déc.	Lisbonne	1er Congrès Portugais de Phitiatrie et Phytopharmacologie	
1984	Avril	Oeiras	3ème Symposium Méditerranéen d'Herbologie	EWRS
1986		Santarém	Journées Systèmes Culturels du Sol de la Vigne	
1991	Mai	Oeiras	Symposium Auxiliaires et Produits Phytopharmaceutiques	
	Déc	Lisboa	Symposium Protection Intégrée du Pommier et Poirier	
1992	Juin	Oeiras	Symposium Solarisation du Sol	
1993	Jan.	Silves	Congrès des Agrumes (Symposium de Production des Agrumes, Symposium de Protection Intégrée des Agrumes	Mairie de Silves, Direction Régionale de l'Agriculture de l'Algarve
	Mai	Lisboa	Colloque de Production Intégrée en Vergers	Association Portugaise d'Horticulture
1994	Jan.	Troia	Réunion Lutte Biologique	
	Avril	P. Lima	Workshop Enseignement de Protection Intégrée	

Welcome adress

Kurt Russ
Bundesanstalt fur Pflazenschutz - Vienna

It is a great honour for me to adress this distinguished audience some words of welcome, and I have to say many thanks to the Organizing Committee for the invitation and the possibility to give this welcome address.

As one who is (or was) working in pest control in common and in fruit fly research in particular since more than 40 years, I am very pleased to be able to participate and to serve in this very important meeting.

If I remember correct it was in 1978 that an IOBC/WPRS working group was set up to deal specifically and actively all the problems which are related to fruit-flies of economic importance. However already during the middle of the sixties and the early seventies the first intensiv efforts were internationaly undertaken to create more and more biological and also integrated methods and strategies to control the various fruit-fly species. I remember, for example, my first contact in this time with President, Prof. Cavalloro during a very interesting meeting on SIT of the IAEA in Vienna.

Ladies and Gentlemen,

Since this time a lot of very important and fruitful work was done not only in Europe but also in many regions round the world, influenced and sponsored by IOBC/WPRS and some solutions and many results related to fruit-fly control were found. Retrospectiv today and especially in this symposium I think, we can see that the research done since more than 20 years was very successful, but also many research will be necessary in future. But nevertheless the IOBC/WPRS can be very proud on the work done in the past all over Europe. This was, and will be a work which helps us not only to reduce pesticide applications but also to minimize danger for man and hazards of environmental pollution.

Ladies and Gentlemen,

the "International Open Meeting" of the IOBC/WPRS working group "Fruit Fly of Economic Importance" which will be opened today is not only a meeting which gives more than 80 scientist of many European countries the possibility to discuss all the problems of their research, but also an excellent event of personell and collegial contacts which were given to all of the participants by the Portuguesien authorities. Many thanks for this.In this sence I wish the meeting very intensiv discussion and many results during the following days and also many stimulations for the research in future.

Welcome adress

J. Piedade-Guerreiro
Chairmam of the "International Open Meeting"

For statutory reasons the Chairman of the Working Group "Fruit Flies of Economic Importance" of the IOBC/WPRS has the competence of organizing every three years a Plenary Reunion that congregates all the experts involved in the fruit flies of economic importance problematic.

We have the joy of having this event taking place for the first time in Portugal and we hope that it occurs with enthusiasm allowing a close and fraternal colaboration between all the experts present here to whom we wish a pleasant stay.

We also wish to express our gratitude to those that made this "International Open Meeting" possible:

To the IICT Presidency and to the IOBC/WPRS our recognition for the facilities given.

To the JNICT we testify our regard for the support rendered.

To the Lisbon Town-Hall we thank fully acknowledge not only for reciving the congressmen but also for lending and setting the technical equipment that greatly contributed for the Meeting's sucess.

To the Media allways ready to divulge this cientific reunions the organizing commitee's gratitude.

And also a final word of thanks for those that anonymously colaborated in the organization and implementation of this Meeting.

We hope that this "International Open Meeting" may contribute efficaciously towards an adequate fight against the fruit flies of economic importance in it's zone of influence - West Palearctic Region.

Session 1

Ecological aspects and biological control of fruit flies populations

Chairman: G. DELRIO
Secretaries: R. JIMENEZ
P. AMARO

Report on Session 1

G. DELRIO; R. JIMENEZ, P. AMARO

At the "Fruit Flies of Economic Importance" Meeting that took place in Lisbon between the 14th and 16th of last October, topics related with ecological aspects and biological control of fruit flies' populations were discussed in the first session. A total of 17 comunications were given by representatives of seven countries of South Europe, North Africa and Argentina. From this papers the following conclusions can be stressed: the need of standardization of the monitorization methods; the necessity of broader analysis of the fruit flies' populations and the well pointed out interest of aspects related with biological control, like the use of natural ennemies and the role of biological insecticides within IPM programs.

NEW MODELS FOR MEDITERRANEAN FRUIT FLY DEVELOPMENT UNDER CONSTANT AND VARIABLE TEMPERATURES

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Summary

We have revisited data on Medfly preimaginal development under constant and variable temperature. Our statistical analyses suggest that the inverse Sharpe & Demichele (1977) rate model fits egg development well. Larval and pupal developments are correctly described by linear relationships. Summation of the three submodels based on duration data may be used to model overall preimaginal development. Extrapolation of our model to natural conditions is discussed.

1 - Introduction

Many mathematical models describe organism growth and development as a function of temperature. Modellers are confronted with several problems of different kinds: the nature of models, the choice of variables, the division of models into submodels, and the type of data collected.

The rate of development through most insect stages is non-linear at low and high temperatures, and linear at intermediate temperatures (Subramanyam et al., 1990). A classical approach for predicting insect development times uses only the linear portion of the curve; this is the degree-day (DD) method with two parameters : the base B and the total number of degrees above B necessary to complete development in a stage. According to the DD concept, time of development under fluctuating temperatures would be similar to that under equivalent constant temperatures with the same total of temperatures. In spite of this restrictive hypothesis the method has been used with success in many instances (Collier & Finch, 1985 ; Subramanyam et al., 1990 ; Fan et al., 1992). However, the linear model does not fit in all natural conditions and more complicated models are needed. Moreover, computers now allow many sophisticated calculations. So the non linear part of the function has been considered and described by several models. Some empirical approaches proposed polynomials (e.g. Tanigoshi et al. 1975) but most models incorporate parameters having a biological meaning (Logan et al., 1976), a biophysical basis (Sharpe & Demichele, 1977), or an ecological significance (Taylor, 1981).

The present paper gives a statistical comparison between several models applied to the Medfly, *Ceratitis capitata*. We assembled the main data published since 1930

giving mean egg, larval and pupal development times at several constant or fluctuating temperatures in laboratory conditions. Our objective is to find the best relationship that could be incorporated in a general model describing population dynamics of the Medfly in natural conditions.

2 - Material and Methods

Data sets

Data are presented in Table I. A preliminary analysis leads to the choice of Messenger & Flitters 's data to model egg development. Bodenheimer and Daza & Criado 's data were chosen for the larval and pupal stages; no data have been found that separated the three larval stages, so larval development was modelled as a whole. In all cases only mean development durations were available and variations in development were at best summarized in standard errors; this information will be used in discussion.

Table I- Bibliographic sources giving the Medfly development as a function of temperature. Two regimes of temperature : c = constant, fl = fluctuating.

Author	Instar	Nutritive medium	Temperature range (°C)	Number of temperatures	Temp. regime
Mc Bride(1934)	egg		12.8, 32.2	2	c
	larval	fruit	12.8, 32.2	2	c
	pupal		12.8, 32.2	2	c
	all		12.8, 32.2	2	c
Bodenheimer (1951)	egg		[12 - 35]	6	c
	larval	artificial	[12 - 35]	6	c
	pupal		[12 - 35]	6	c
	all		13.8	1	c
Messenger & Flitters (1958) (1959)	egg		[11.4 - 35.8]	19	c
	egg		[11.1 - 32.2]	15	fl
Hooper (1978)	egg		20.2, 25.1, 29.8	3	c
	larval	artificial			
Shoukry & Hafez (1979)	egg		[22 - 35]	6	c
	larval	artificial	[17 - 35]	7	c
	pupal		[22 - 32]	5	c
Ros (1982)	larval	orange	12.4, 25.5	2	c
	pupal		11.4, 21	2	c
	total	orange	13.8, 22	2	c
Daza & Criado (1984)	egg		[19 - 31]	5	c
	larval	artificial	[19 - 31]	5	c
	pupal		[19 - 31]	5	c
Rivnay (1991)	larval	grapefruit	22, 26	2	c
		fig	18.5, 29	2	c
	pupal		[15 - 29]	4	c
Mourikis (1965)	egg		[19 - 32]	12	c
	larval	peach	24, 30	2	c

The linear model

In the degree-day (DD) model two parameters are estimated: the base B and the thermal constant: $K=1/\alpha$ (α=slope of the linear relationship). We use Schaffer 's (1983) formula to estimate a confidence interval for K from the standard deviation SD of α:

$$SD(K) = SD(\alpha) / [\alpha^2 - SD^2(\alpha)]$$

To choose which variable must be modelled, rate or duration, duration data were also fitted to the inverse model of the linear model : duration = $1 / (c_0 + c_1.T)$, where T is the temperature (in °C).

Non-linear models

We choose the Logan et al. (1976) and Sharpe & Demichele (1977) models because of their biological or biophysical bases. The first incorporates two distinct mechanisms: the development rate increases exponentially from some base to optimum temperature; once that value has been exceeded the rate decreases precipitously up to the lethal temperature. For example a logistic and an exponential are usually combined in the model:

$$v(T) = v_m / [1 + k.\exp(-rT) - \exp(-\tau)] \qquad (1)$$

with : v=the development rate, r=the composite Q_{10} value for critical enzyme-catalyzed biochemical reactions, $\tau = (T_m - T)/dt$, T_m=the temperature above which no further development occurs, dt=the width of the upper boundary in which the rate begins to decline rapidly, v_m=the maximum observed rate, $k=(v_m - v_0)/v_0$ with v_0=the rate at the lower threshold temperature.

Since parameters have a biological meaning, initial estimates can easily be found. The model accounted for asymmetry about optimum temperature and was efficient for describing systems operating at or above optimum temperatures.

Sharpe & Demichele (1977) and Sharpe et al. (1977) have built a biophysical model that describes the entire response curve over a large range of temperatures. Their model is derived from the Eyring equation assuming multiple activity states of the underlying developmental control enzymes. Enzyme inactivation at low and high temperatures has a compensatory effect. We used Schoolfield et al. 's (1981) modification of the formulation which facilitates the estimation of parameters with non-linear regression techniques:

$$v[T] = \frac{\rho_{25} \cdot \frac{T+273.15}{298.25} \cdot EXP\left[\frac{\Delta H_A}{R} \cdot \left(\frac{1}{298.15} - \frac{1}{T+273.15}\right)\right]}{1 + EXP\left[\frac{\Delta H_L}{R} \cdot \left(\frac{1}{T_L} - \frac{1}{T+273.15}\right)\right] + EXP\left[\frac{\Delta H_H}{R} \cdot \left(\frac{1}{T_H} - \frac{1}{T+273.15}\right)\right]} \qquad (2)$$

R=the universal gas constant (1.987 cal/deg/mol), ρ_{25}=the development rate at 25°C assuming no enzyme activation, ΔH_A=the enthalpy of activation of the reaction

that is catalyzed by the enzyme, T_L=the temperature at which the enzyme is half active and half low temperature inactive, ΔH_L=the change in enthalpy associated with low temperature inactivation of the enzyme, T_H and ΔH_H=the same as T_L and ΔH_L but for high temperatures. All six parameters have graphical interpretations. Equation (2) may be reduced to 4 parameters to remove inactivation at low or high temperature; this new model is useful when data are scarce.

Initial estimates were provided by MATHEMATICA package (version 2.0).

The non-linear models were fit to the data sets using SAS (PROC NLIN, SAS Institute, Version 6) and the Marquardt algorithm. In some cases least squares were weighted by 1/rate or $1/(\text{rates})^2$ according to the variability of data. When we worked on the duration of development the inverse of equation (2) was used. Data sets with more than eighteen temperatures allow the estimation of the six parameters of Sharpe & Demichele 's model; otherwise the four parameter model was used.

Fluctuating temperatures

Fluctuating temperatures in the laboratory usually followed a sinusoidal scheme defined by an amplitude and an average value. We used a modified version of Hagstrum & Milliken 's (1991) package to fit our models. No satisfactory solution was available for graphical representation at fluctuating temperatures; as suggested by Roltsch et al. (1990), rates or durations were plotted against average temperature values.

Model validation and comparison

The fit of each model was assessed by analysis of the residuals between data and estimates; serial correlation of residuals was detected by the Durbin-Watson (DW) test (Draper & Smith, 1981). Models were compared on their normalized residuals: the best model has the lowest values. The coefficient of determination R^2 was not used since it can be very misleading in non-linear regression (Kvalseth, 1985).

3 - Results

Egg development

Logan et al. 's model applied to the rate of development fitted the Messenger & Flitters 's data well at high and intermediate temperatures but poorly at low temperatures (Fig. 1A). Weighting the least-squares estimation with 1/rate slightly decreased correlation between parameters (Table II). Using duration development as variable also led to a good fit (Fig. 1B). Plots of the normalized residuals against temperature showed the same sinusoidal trend whatever the variable used (rate or duration) (Fig. 1C). The Durbin-Watson statistic was unfortunately inconclusive.

The fit of rate data to Sharpe & Demichele 's model (SD) is excellent (Fig. 1E); parameter estimations are given in Table III for the unweighted and weighted (1/rate)

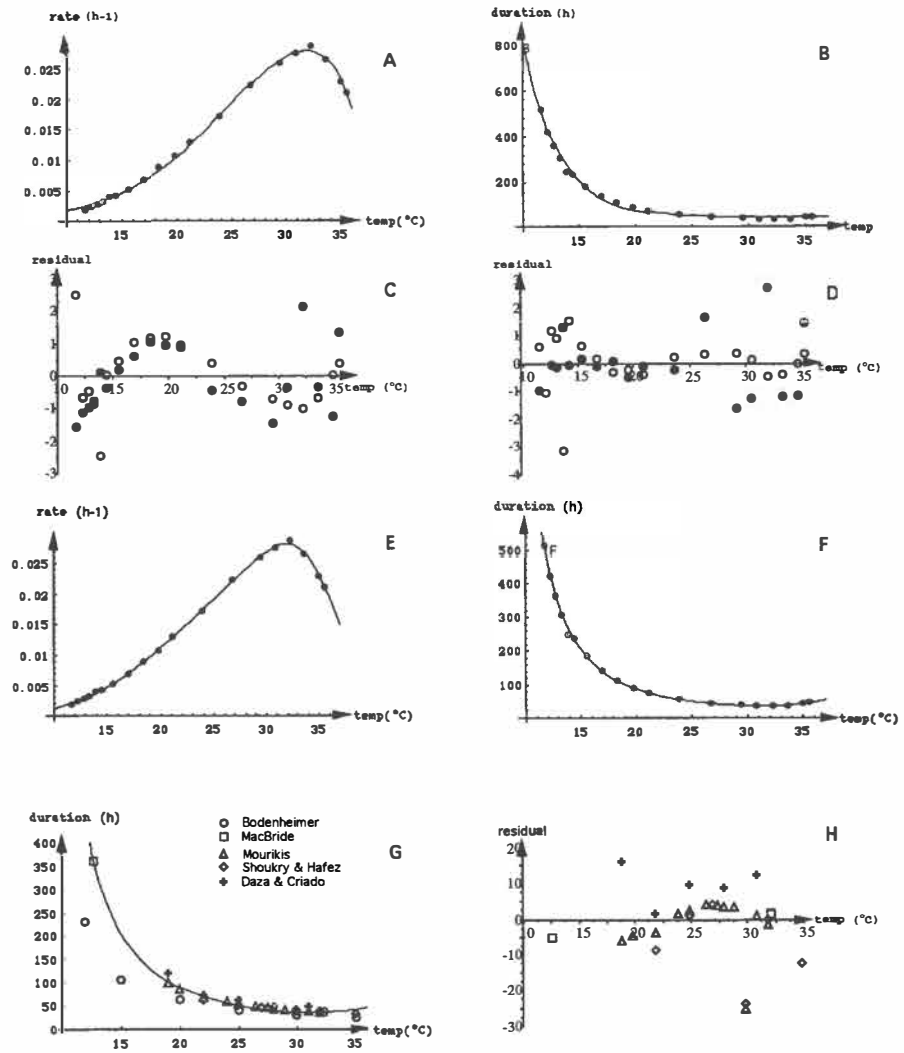

Fig. 1 - Egg stage development at constant temperature - Messenger & Flitters's data.
A and E : rate data fitted by Logan et al (1976) and Sharpe & Demichele (1977) models.
B and F: duration data and above models inverted.
C and D : normalized residuals against temperature, full circles for models A and E, empty circles for models B and F.
G : data of McBride (1934), Bodenheimer (1951), Mourikis (1965), Shoukry & Hafez (1979) and Daza & Criado (1984) are superposed with model B.
H : raw residuals for data sets of figure G except Bodenheimer.

Table II - Parameter estimation for Logan et al. 's weighted (1/rate) and unweighted model

parameter	v_m	k	T_m	dt	r
unweighted	.038073	140.616431	38.081910	2.402202	.201000
weighted	.032761	188.857819	37.568679	1.713595	.228318

models. Normalized residuals are randomly distributed when plotted against temperature (Fig. 1D); the non significant value of the DW statistic implies that no serial correlation exists between the residuals; their absolute values are on the average lower than in Logan et al. 's model. Weighting does not improve the fit (Table III). Adjustment of duration data to the inverse of equation (2) is also excellent (Fig. 1F and D). The difference between the two models is the higher variability of residuals at elevated temperature in the inverted model.

Table III - Sum of squares (Sum) and estimates of parameters for unweighted and weighted (1/v) Sharpe and Demichele rate model and for the inverse model (time).

Parameters	Rate Model		Duration Model
	unweighted	weighted	unweighted
Sum	7.3 10-7	5.9 10-5	283.65
$\rho 25$	.02127	.02035	.02142
ΔH_A	10762.52898	12608.14509	20931.18702
T_H	308.82414	308.57837	305.90760
ΔH_H	97564.57369	90938.83626	59387.51399
T_L	289.13185	287.98814	284.85606
ΔH_L	-43449.96477	-48008.28899	-70752.87434

Of the other data sets, two (McBride and Mourikis) validated the SD model (Fig. 1G, 1H); two give a less good fit: Shoukry & Hafez, and Daza & Criado, but only one temperature lower than 19°C has been tested in these experiments. Conversely data from Bodenheimer fitted badly to SD model (Fig. 1G).

At fluctuating temperatures (Messenger & Flitters, 1959) the fit was bad especially at temperatures lower than 15 °C (Fig. 2A). Adjusting data duration directly lead to a better fit (Fig. 2B).

Models were compared from the normalized residuals computed for three temperatures, two extreme and a medium (Table IV). The inverse model of Sharpe & Demichele gave the lowest residuals (in absolute value) whatever the temperature and the Logan et al. with an inverse-rate ponderation, the highest. However all the residuals were non significant, implying that all the models selected are acceptable.

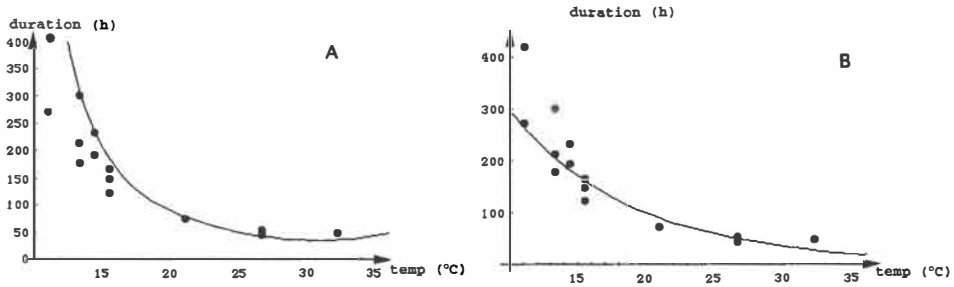

Fig.2 - Egg stage - fluctuating temperatures - Messenger & Flitters's data.
A : data superposed to model figure 1F.
B : data superposed to Hagstrum model with six parameters.

Table IV - Comparison of normalized residuals for three constant temperatures (two extreme and one median) for different models. 6p = model with 6 parameters, 1/v = weighting by the inverse of rate v.

Model	11.67°C	23.89°C	35.56°C
Sharpe (6p)	-0.979	-0.185	0.906
1/Sharpe (6p)	0.2677	0.231	0.263
Logan (1/v)	-1.590	-1.650	0.727
Logan ($1/v^2$)	-0.954	0.294	0.763

Larval development

Whatever the data set, the rate of development increases linearly with temperature (Fig. 3A); however the slope of the relationship depends on the set. Bodenheimer and Daza & Criado 's data were grouped and fitted to a straight line:

$$v(T) = 0.001\ (0.3451\ T - 3.38) \qquad R^2 = 0.98 \qquad (3)$$

The slope and the ordinate at the origin are highly correlated (r = 0.90). K (=1/slope) was estimated at 120.77 DD (basis 9.8°C), with limits of confidence interval (109.4 and 131.17). When duration data were fitted to the inverse of a linear model (Fig. 3B), parameter estimations differed slightly:

$$d(T) = 1000/(3.1543\ T - 3.00) \qquad (4)$$

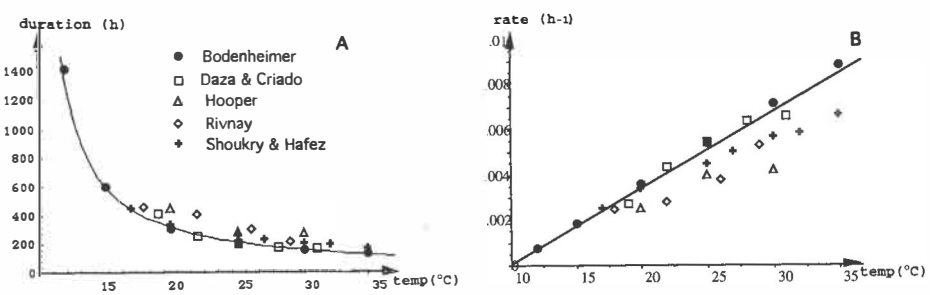

Fig.3 - Larval stage - Five data sets. The Bodenheimer and Daza & Criado data only are fitted to a duration model (A) and a linear rate model (B).

Model 3 was compared to Bodenheimer 's estimations: K = 117.8 (basis 9.8°C). The latter led to smaller values, especially at low temperatures: at 15°C the larval stages would last 19.1 days according to Bodenheimer and 22.7 days from (3).

The other data sets (Rivnay, Hooper and Shoukry & Hafez) did not fit models 3 and 4 (Fig. 3A, B). Differences in the nutritive medium (synthetics or various fruits) probably explain the discrepancies.

Pupal development

A linear equation described the relationship between temperature and pupal rate for Bodenheimer and Daza & Criado data (Fig. 4A):

$v(T) = 0.001\ (0.2390\ T - 2.34)$ $\quad R^2 = 0.98$ (5)

Adjustment to the duration data lead to similar values of parameters (Fig. 4B):

$d(T) = 1000/(0.2111\ T - 2.00)$ (6)

McBride, Ros and Rivnay data validated models 5 and 6.

Global model

A global model incorporates all the above models calculated from duration data: the inverse model for egg development, models 4 and 6. By summing the three models a good agreement is observed with the three data sets available (McBride, Bodenheimer and Ros) (Fig. 5).

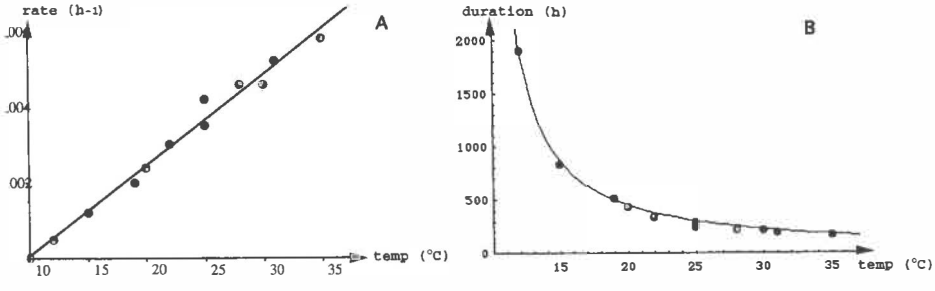

Fig.4 - Pupal stage - Bodenheimer and Daza & Criado data fitted to a linear model (A) or to the inverse model (B)

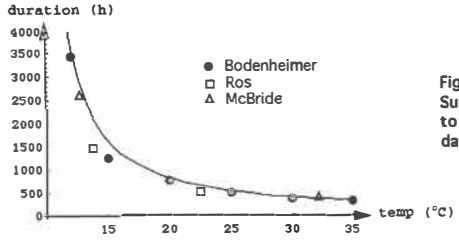

Fig. 5 - Global model
Superposition of the global model (from egg to adult) on McBride, Bodenheimer and Ros data.

4 - Discussion

Our statistical analyses are more sophisticated than the previous applied to the Medfly. We find that fits to the data sets are usually improved by our non-linear models, especially for egg and overall development.

The assumptions of a linear temperature-rate model are not valid for egg development and the best model is the inverse equation of Sharpe & Demichele (1977) applied on duration data (inverse of equation 2). Conversely, larval and pupal developments are correctly described by linear models except that the parameters for the larval stages depend on the diet given to larvae. Discrepancies between Bodenheimer - Daza & Criado data and Hooper - Rivnay - Shoukry & Hafez data are large and may reach 2 days at 20 °C. A more complete model should incorporate another parameter such as sugar or pectine content of the larval medium or the degree of fruit maturity. The overall model applied from oviposition to emergence is in good agreement with the three data sets available. Although the equation is complex no simpler model was searched for. The fact that the addition of three submodels (egg, larva and pupa) leads to a valid model, suggests that the rates of development for the three stages interact only slightly. Of course genetic variation exists in Medfly but is overrun by temperature effects.

The applicability of our models to fluctuating temperatures must be questioned. Variable temperatures may be simulated in the laboratory or recorded in the field. Our statistical analysis shows that the model (Fig 1F) proposed at constant temperatures works badly at fluctuating temperatures below 15°C. However the conclusion comes from a single data set (Messenger & Flitters) and only for egg development. In mass production of the Medfly larval rearing procedures vary with respect to temperature. For instance, in Mexico, cultures are incubated at 30°C for 36 hours, then larvar are transferred at 27°C and finally pupal development occurs at 22°C (Vargas, 1989). It would be interesting to test our model on the corresponding data. One very useful improvement for mass rearing would be the incorporation of individual variability in development into the model.

Our model should also be tested on field data. As yet no general rule emerges for the applicability of non-linear models to field conditions; this mainly depends on the species under study, on the range of temperature, on the quality of temperature measurements and on the effects of environmental factors other than temperature on insect development (Hochberg et al., 1986; Hagstrum & Milliken, 1991). Of course we need field-collected development data for individual egg cohorts to test our model.

Acknowledgments

We thank J.C. Hervé for discussions on the non-linear models and R. Grantham for comments and help with our English.

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UNFECUNDATED DATES, HOST OF THE MEDITERRANEAN FRUIT FLY (DIPTERA:TEPHRITIDAE) IN THE OASES OF TOZEUR, TUNISIA

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SUMMARY

In the oasis of Tozeur in the south-western part of Tunisia, unfecundated dates of the Deglet Nour variety were found in November infested by the Mediterranean fruit fly, Ceratitis capitata (Wiedemann). In the Tozeur Governorate, crops are confined to oases in which by far the most abundant fruit tree is the date palm. Although the date palm is known not to be one of the preferred hosts and ripe soft dates are not infested, the development stage preceding maturation when the fruit turn from green to yellow or red is suitable for oviposition and larval growth. This also is true for unfecundated dates becoming yellowish.

Even though Medfly infestation is occasional, the importance of the date palm as host plant in North Africa is related to its large numbers in palm groves and the occurrence of the stage in dates suitable for oviposition when most of other fruit has ripened and been harvested.

INTRODUCTION

Since 1988, Tunisia is participating with the three other North African countries (Algeria, Libya and Marocco) and the technical assistance of the International Atomic Energy Agency (IAEA), in a reginal programme based on the sterile insect technique (SIT), for the control and eradication of the Mediterranean fruit fly, Ceratitis capitata (Wiedemann) (Diptera: Tephritidae) (Klassen, Lindquist, Buyckx, 1993; Buyckx, 1993), so far the only polyphagous fruit fly found in the Maghreb. The second phase of this Programme started in January 1993 and consists mainly in pilot trials to train national staff and adapt SIT operations to local conditions. The area of Tozeur has been selécted as a first pilot trial for its natural isolation and relatively small cultivated area. Located in the south-western part of Tunisia, between 7°55' and 8°26' East and 33°50'-34° North, the Governorate of Tozeur is part of the very arid subclimatic zone of North Africa,

with an average annual rainfall of 100 mm and mean annual temperature of 27° C. Of its total area of 559.287 ha, 45% is steppe or jerid, 40% muddy salt flats or chotts at 16 m above sea level, the jebel or mountain covering the remaining surface. The steppe bears a diffuse vegetation consisting mainly in perennial grasses with some rhamnaceous and mimosaceous scrub, and tamarisks. Crops only grow in oases irrigated with underground water and in the jebel el Negaeb range by a few oueds. The irrigated area consistsin 26 oases totalling about 8.000 ha, the largest being the Tozeur oasis (1.100 ha) at 51 m above sea level.

The Ministry of Agriculture has undertaken Medfly trapping in the Tozeur area as part of a nationwide network and started fruit infestation studies to obtain data on the pest distribution and population fluctuations.

This paper is an attempt at understanding the role of the date palm and other host plants in Medfly reproduction in the Tozeur oases.

THE DATE PALM (PHOENIX DACTYLIFERA L.), HOST OF THE MEDFLY

In the FAO study on date production and protection (1982), the Medfly is listed among mites and insect pests of minor economic importance. During a visit of the Tozeur oases end November 1992, the author found no Medfly larvae in mature dates harvested or collected at the foot of the palms. However, out of a dozen unfecundated, yellowish dates picked from a Deglet Nour palm, four were found to contain from one to three fruit fly larvae. Reared in these dates at the Entomology Unit of the IAEA Agricultural Laboratories at Seibersdorf, Austria, the larvae pupated and produced Medflies (J. Hendrichs, personal communication). So far infestation of dates by this fruit fly had not been reported from Tunisia. Dhouibi (1991), reviewing the main pests of date palm and dates in Tunisia, most of his field observations concerning the Tozeur palm groves, does not mention the Medfly, although (personal communication) he did find occasionally Medfly larvae in dates rotting on the ground. He reports brreding of the carob moth, Ectomyelois ceratoniae (Z), in unfecundated dates in Tozeur.

A few cases of Medfly infestation of dates have been reported from other Maghreb countries and other continents, as mentioned by Lepesme (1947) in his review of insects of palm trees. Langronier (1941) reporting the presence of the Medfly on citrus and stone fruit in the oases of Berriane, El Golea and Laghouat, Algeria, drew attention to the risk that dates also would be infested. Sacantanis (1955, 1957) lists the date palm as semi-wild host in Morocco, without mentioning any breeding in dates which so far are not considered as host in this country (Bounfour, 1992). In the coastal area of Libya, Black et al (1987) collected in September ripe dates from palms of two local varieties and

found fruit of the Karkabi variety, blackish brown with black punctures, infested; out of a 2 kg sample only a "few" Medflies were obtained. The dates of the Hallaawi variety were fully ripe but yellow with many brown spots, and although weighing only 0.5 kg, the sample yielded a "large" number of Medflies. The authors concluded the Hallaawi variety was more attractive for the pest than the other due to its higher sugar content.

In Hawaii, Back and Pemberton (1918a, 1918b) reported to have found only one instance of Medfly infestation in green well-grown dates of an unimproved date palm; thirty-five dates yielded only two adults. The authors considered this as probably a chance infestation and for practical purposes dates to be immune. In Palestine, Bodenheimer (1930) also found the pest in dates and later (1951) having obtained only a few flies from this fruit, stated that the date palm is not a regular host. However Avidov and Harpaz (1969) ranking this palm tree among the host plants in this country, give it the same low infestation index (1-2) as for prickly pear, Opuntia ficus-indica L. (Miller). Eskafi and Cunningham (1987) listed dates among those fleshy fruits in Central America in which C. capitata was not found. In Southern Egypt, Hendrichs and Hendrichs (1990) listed the date palm among possible nonhost of the Medfly, in the absence of reports on date infestation. Observing in September and October fly distribution among fruit trees in and around an irrigated orchard, they noted very few flies were present on vegetation surrounding it but sighted a few on dates palm practically all growing outside the orchard.

Other species of fruit flies have been reared from dates (White and Elson-Harris, 1992): the Caribbean fruit fly, Anastrepha suspensa (Loew), Bactrocera (Bactrocera) sp. B. dorsalis (Hendel); in Queensland B. (B.) neohumeralis (Hardy) and the Queensland fruit fly, B. (B.) tryoni (Froggatt); in Pakistan the peach fruit fly, B. (B.) zonata (Saunders).

DISCUSSION

It is obvious that during the handling, sorting and packaging of ripe and marketable dates the presence of Medfly larval infestations would have been noted, as it is the case for pyralid pests, mainly the carob moth infesting dates in bunches in groves as well as in storage, and causing economic losses in the Tozeur area (Dhouibi and Jarraya, 1988; Dhouibi, 1991). The apparent contradiction in authors' reports on reproduction of the Medfly in dates may find an explanation in the stages of development of the fruit as described in the FAO paper (1982). The first stage known as habbabōk, is a round creamy white berry of about 0.5 cm diameter, becoming in

the next stage called chimri (khalaal akhdhar) a green and hard oval berry between 3 and 4.5 cm in length. In the following stage, khalaal, the fruit is becoming larger, more oblong, still hard but turning yellow or red. During the last ripening stage or rutab, the date becomes an oblong berry with a fleshy pericarp and a terminal stigma, generally brown or dark brown, yellow in certain varieties. Depending on the variety and the growing conditions, it varies in weight from about 2 to 60 g, in length from about 1.8 to 11 cm and in width between 0.8 to 3.2 cm. With wind pollination not all female flowers become fecundated. To obtain good yields, artificial pollination is indispensable, which in Tozeur starts in mid-March until mid-May. Generally in the later part of June dates reach the chimri stage and in July they turn from green into yellow passing in the khalaal stage characterized by the enzymatic transformation into sugar. Certain early varieties are ripening already in July, whilst Deglet Nour is late maturing and ripe only end October-November. Growth of unfecundated dates is slower, reaching and stopping at the early khalaal stage and for the Deglet Nour variety turning yellow in November. As from December, rutab and unfecundated dates start drying out in the bunches remaining on the palms and on the ground.

It is likely that dates become suitable for oviposition during the khalaal stage and unfecundated dates later in this period due to their slower growth. Infestation data are lacking on the end of the chimri stage and on the khalaal stage for Deglet Nour and the main varieties to determine varietal susceptibility, as indicated by the Libyan study (Black et al.,1987). The development of the dates extends over the hottest months, the period June to October, during which the short life cycle allows seven generations of the Medfly (Table 1). Whilst infestation of dates may be slight, as reported by several authors, and of no economic value, the importance of the date palm as host plant in an environment hostile for the Medfly (Buyckx, 1993) is related to: (1) the time dates reach a stage suitable for oviposition when other fruit has ripened and been harvested (Table 3) and (2) to their numbers. In Tozeur oases the most abundant fruit tree is the date palm (Table 2). Many varieties are grown - Kearny (1906) has described 63 for Tunisia - but 56% of the palms belong to the commercially most interesting Deglet Nour variety. According to the Coachella Date Growers Institute (1941), the number of dates on bunches of 12-year old Deglet Nour palms near Indio, California, was from 288 to 1,406. Assuming

Table 1. TEMPERATURES AND MEDFLY DEVELOPMENT IN TOZEUR, TUNISIA

Month	M*		m*		Development in days	
	1991	1992	1991	1992	1991	1992
June	35.04	34.50	21.88	21.80	18.7	19.0
July	38.90	36.89	25.20	24.29	15.7	16.7
August	38.27	38.43	25.53	26.21	15.8	15.5
September	34.92	34.80	23.34	23.30	18.0	18.2
October	28.81	30.05	18.12	18.62	25.7	24.1

M: monthly mean of dayly maximum temperatures

m: monthly mean of dayly minimum temperatures

*: data provided by Merhaben, J. (personal communication)

an average of 850 dates per bunch, three bunches per palm, in an oasis of 100,000 palms there would be 255 million dates reaching the khalaal stage between end June and August. If only 0.1% of them had only one larva, the potential production would be 250,000 flies. Even if infestation is occasional, the large number of palms present at high density in an ancient, typical palm grove could support quite a large Medfly population just before passing the cool winter, to infest other fruit when it reaches maturity in spring (Fig.1).

OTHER HOST PLANTS

In the Tozeur Governorate, in total about 260,000 fruit trees of which over 150,000 in production (Table 2), are scattered over the variuos oases; in addition, there are grape stocks, about half of them producing fruit. Water being in short supply and paid for, irrigated soil is exploited as much as possible. In ancient oases three vegetation levels may be distinguished, each one including host fruit for the Medfly. At ground level, vegetables such as tomatoes, chili peppers etc. are produced, as well as fodder crops, mainly luzern. The middle level consists of pomegranates, stone- and pomefruit, citrus and figs. The palm crowns are the upper level and due to the high density palms were planted some centuries ago, form a canopy protecting from the hot and dry wind from the desert. Over the last three decades, modern oases have been established with Deglet Nour only at densities of 100 or 156 palms per hectare, leaving light and space to grow vegetables, cucurbits and fruit trees. The fruit produced in the Tozeur area include hosts preferred

Table 2. NUMBER OF FRUIT TREES IN TOZEUR GOVERNATE, TUNISIA

	in production	total
Date palm	-	1,390,000
Pomegranate	46,790	81,910
Apricot	25,044	55,620
Peach	34,100	53,640
Citrus	21,800	24,800
Apple	11,610	22,000
Fig	6,400	11,880
Olive	5,330	6,050
Prune	1,542	4,332
Pear	1,260	2,430

* After Merhaben, J. (1992)

for oviposition by the Medfly, namely apricot, fig, peach and pear. In certain oases some type of fruit is predominant, as for citrus in Mides. It should be noted that the semi-wild prickly pear, widespread in Tunisia and infested mainly in fall is absent from the area, except for Tamerza oasis.

As far as wild plants are concerned, the natural vegetation of Central Tunisia (Long, 1954) extending into Tozeur Governorate, includes the following host species:

Rhamnaceae (buckthorn): Rhamnus alaternus L. ssp. myrtifolia (Willk.) Maire, Rhamnus lycioides L., Rhamnus lycioides L. ssp. oleoides (L.) Jahan. & Maire, Ziziphus lotus (L.) Lam. (lotus).

Solanaceae: Lycium europaeum L. (box thorn, matrimony vine).
Capparidaceae: Capparis spinosa L. (caper)

Zygophyllaceae: Nitraria tridentata Desf. (nitre bush?)

In November 1992, the author found ripe fruit of R. alaternus ssp. myrtifolia and of Z. lotus infested in the Degache oasis. Further, C. capitata has been reared from L. subglobosum in Northern Tunisia (Soria, 1962) whilst for Bodenheimer (1951) L. europaeum is a doubtful host. Black (1992) reports to have obtained Medflies from ri-

Table 3. RIPENING PERIODS OF FRUIT GROWN IN TOZEUR, TUNISIA, SUITABLE FOR OVIPOSITION BY THE MEDFLY*

Host fruit	J	F	M	A	M	J	J	A	S	O	N	D
Khalaal dates							———	———	———	———		
Unfecundated dates							———	———	———	———	———	———
Pomegranates									———	———	———	———
Apricots					———	———						
Peaches						———	———	———				
Citrus	———	———	———	———	———	———				———	———	———
Apples							———	———	———			
Figs							———	———	———			
Prunes					———	———	———					
Pears							———	———	———			
Grapes								———	———			

* After Merhaben, J.(1992)

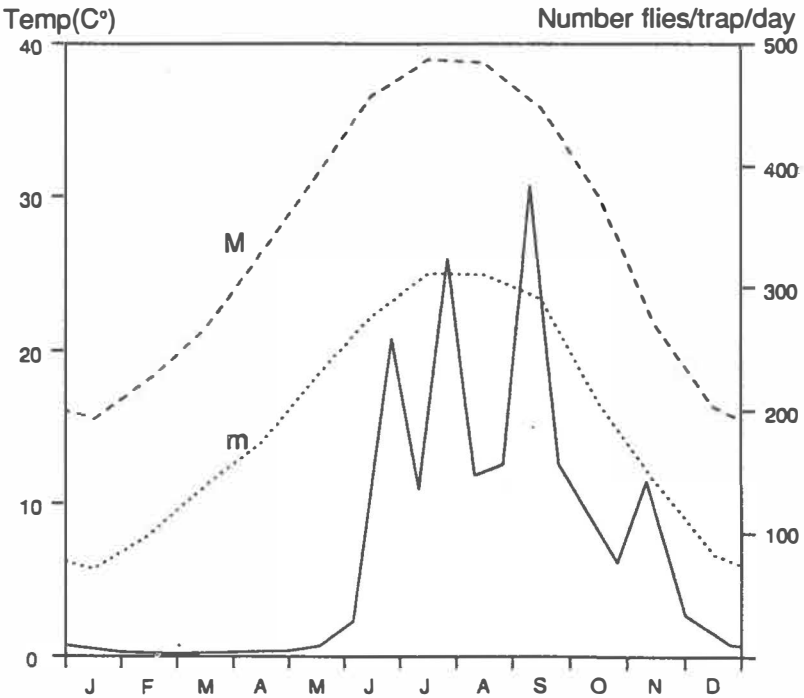

Fig. 1. Degache oasis, Tozeur- trap catches over a twelve months period from July 1992 to June 1993, and monthly means of daily maximum (M) and minimum (m) temperatures for the period July 1991 to June 1992.
Source: Merhaben, J. (1993)

pe fruit of N. retusa in the Zawia area of Libya. Although caper is listed in Libya as host of the Medfly, this requires verification for Tunisia. Whilst these wild hosts,scarcely distributed in the steppe, bear few fruit shrivelling up quickly, they can produce, most of them during September to November, a lot of berries suitable for larval development when water is available in oueds and irrigated soil where some are planted as hedges.

As shown in table 3, the period of greatest fruit availability for the Medfly in Tozeur palm groves starts in June and terminates in November. From December to April, only citrus can be found, including sour orange, Citrus aurantium L., but relatively low temperatures prevailing during the winter months greatly reduce the activity of the Medfly. Trap catches over a twelve months period in an ancient palm grove clearly show the effect of host availability and temperature (Fig. 1).

CONCLUSIONS

It is likely that dates contribute with pomegranates and oranges to maintaining in Fall a Medfly population which after the cool Winter starts breeding in late maturing oranges and early varieties of apricot and prune, then to continue on peaches, figs, pears and apples. Fruit infestation studies will determine the role of the various host species in the population build-up and show the importance of apricots, peaches and figs. Dates can support breeding of the fly. Their importance as host is related to the large numbers of date palm in the groves and the occurrence of a stage suitable for oviposition when most of other fruit has ripened and been harvested.

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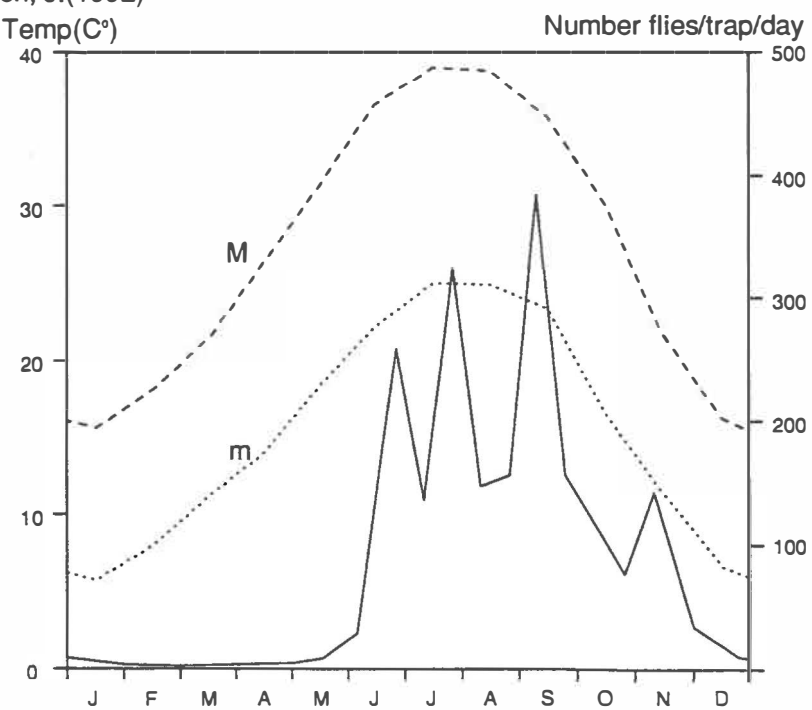
Khalaal dates							———	———	———	———		
Unfecundated dates							———	———	———	———	———	———
Pomegranates										———	———	———
Apricots					———	———						
Peaches						———	———	———				
Citrus	———	———	———	———	———	———				———	———	———
Apples							———	———	———			
Figs							———	———	———			
Prunes						———	———					
Pears							———	———	———			
Grapes								———	———			

* After Merhaben, J.(1992)



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M: monthly mean of dayly maximum temperatures

m: monthly mean of dayly minimum temperatures

*: data provided by Merhaben, J. (personal communication)

Etude de l'activité de la cyromazine sur le developpement de *Ceratitis capitata* Wied. (Diptera, Tephritidae): mortalité et anomalies morphoanatomiques

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D'aprés l'étude des effets sur la fécondité et fertilité de la mouche mediterranéen des fruits qui a mis en évidence une réduction important dans le nombre d'oeufs pondus ainsi comme dans le pourcentage d'eclosion et pourtant dans le nombre de larves obtenues, nous avons étudié les effets de l'adittion de la cyromazine dans la nourriture de la femelle, sur le développement des individus obtenus des oeufs pondus par ces femelles.

L'objectif général de l'étude présenté est d'établir si l'adittion de cyromazine voie alimentaire a des effets sur le développement, anatomie et mortalité des larves obtenues des oeufs pondus par les femelles *Ceratitis capitata* Wied. alimentées avec une diete traité et si ces effets sont concentration dependants.

L'étude a été porté sur 30 couples et trois repetitions. Quatre concentrations de cyromazine ont été testées: 10 mg/ml, 0,1 mg/ml, 0,01 mg/ml et 0,00001 mg/ml.

Les paramètres considérés pour chaque femelle et ponte sont: le pourcentage d'éclosion, l'évolution temporel du développement, la mortalité et la présence ou non d'anomalies morpho-anatomiques chez la larve, la pupe ou l'adulte.

Effets sur le nombre d'oeufs pondus

Nous avons trouvé une réduction du nombre d'oeufs pondus, c'est à dire, du potentiel reproducteur pour les concentrations de 10 et 0,1 mg/ml. Le nombre d'oeufs pondus pour les autre concentrations ne differs pas du témoin. Nous pouvons conclure que l'effet sur le nombre d'oeufs pondus est concentration dependent.

Effets sur l'éclosion des larves

Le pourcentage d'eclosion de les larves differs de façon assez important entre le témoin et les traités avec 10 mg/ml. Cet pourcentage est, en moyen, 89% dans le témoin tandis que pour la concentration de 10 mg/ml varie entre 48-56%. Pour les autre concentrations testées nous n'avons pas trouvé des differences.

Effets sur le développement des états preimaginaux

L'effet plus important trouvé sur le développement des larves est une grande mortalité des jeunes larves obtenues des oeufs pondus par des femelles alimentées avec la cyromazine pour la concentration de 10 mg/ml. Seulement un 17,7% des larves obtenues ont montré un développement larvaire normal. Le 82,3% meurent just aprés l'emergence.

Nous n'avons pas trouvé des differences dans la durée du développement pour aucune des concentrations testées. Nous n'avons pas trouvé non plus aucun effet sur l'anatomie des états preimaginaux. Dans tous les cas étudies on trouve un petit nombre (entre le 7-10%) d'indivius avec, bien une emergence incomplete, bien des malformations, mais on trouve aussi ces malformations dans le témoin.

Effets sur la pupation

Nous n'avons pas trouvé des effets sur la pupation sauf pour la concentration de 10 mg/ml. Pour cette concentration seulement un 0,3 % des larves survivants ont formé le *puparium*. Pour les autres concentrations

testées et pour le témoin le pourcentage de pupation varie entre un 67% et un 82% selon la répétition.

Effets sur l'émergence des adultes

Nous avons trouvé des effets trés importants sur l'émergence des adultes, effets qui se montrent concentration dependent. Pour le témoin le pourcentage d'émergence est en moyenne de 76%. Pour la concentration plus bas (0,00001 mg/ml) le pourcentage trouvé est 64% et differs significativement du témoin. Pour les concentrations de 0,01 et 0,1 mg/ml le pourcentages obtenus sont 56% et 53% respectivement. Ce pourcentage est sensiblement plus bas que ce du témoin et differs aussi du pourcentage obtenu pour la concentration de 0,00001 mg/ml. Pour la concentration de 10 mg/ml nous n'avons pas obtenu aucun adulte.

D'aprés ces resultats nous pouvons conclure que l'aplication de la cyromazine dans la diète des adultes a des effets importants sur le nombre d'adultes obtenus de sa progenie, et que ces effets son concentrations dépendants.

En conclusion, la cyromazine ajouté a la nourriture des adultes de *C. capitata* a des effets trés importants sur le développement de sa progenie. Il existe une légere réduction du nombre d'oeufs pondus accompagne d'une réduction dans le taux d'éclosion des larves et d'une grande mortalité des jeunes larves pour la concentration de 10 mg/ml. En plus, il existe pour cette concentration une important mortalité aussi pour les larves plus agées et une réduction presque jusqu'á cero du taux de pupation. Ces effets ont pour conséquence l'élimination de toute la progenie des adultes alimentés avec la concentration de 10 mg/ml.

Pour les autres concentrations testées les effets sont beaucoup moins importants sur la pupation. L'alimentation des adultes avec ces concentrations provoque sourtout une diminution dans le nombre d'adultes obtenu (entre 40-50% de réduction) comme conséquence sourtout des effets sur l'émergence des adultes, effet qui comme on a vu est concentration dépendant.

Fluctuations des populations naturelles de la mouche méditerranéenne des fruits *Ceratitis capitata* Wied. (Diptera Trypetidae) dans deux biotopes du Nord de la Tunisie et évolution de l'infestation des *Citrus*

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Résumé

Au terme de deux années d'étude sur l'activité de vol de la mouche méditerranéenne des fruits dans les biotopes de Mraïssa et Raf raf du Nord de la Tunisie, des données sont disponibles sur le nombre de générations annuelles du ravageur ainsi que sur l'infestation des fruits de *Citrus*.

Dans chacun des deux biotpoes d'étude, les fluctuations de la population naturelle sont similaires pour les deux années 1991 et 1992, le maximum de capture est obtenu en septembre-octobre pour l'année 1991 et en novembre-décembre pour l'année 1992 en raison vraisemblablement de la variation des conditions climatiques. Le niveau de la population serait corrélé avec le maximum d'infestation des fruits hôtes.

INTRODUCTION

La mouche méditerranéenne des fruits *Ceratitis capitata* Wiedemann 1824 est l'une des mouches des fruits les plus redoutables. De par sa très grande polyphagie d'où découle une large gamme de plantes hôtes (DEBOUZIE, 1980), la cératite possède une faculté d'adaptation remarquable, un potentiel biotique élevé (HARRIS, 1977) et une capacité remarquable de sélectionner les fruits hôtes (BODENHEIMER, 1951 ; FERON, 1962 ; BATEMAN, 1972 ; 1974 ; HENDRICHS & HENDRICHS, 1990) .

En Tunisie, la cératite est probablement la plaie la plus répandue dans les différentes contrées fruitières, particulièrement les fruits d'été et les *Citrus*(DRIOUCHI 1990) .

Nous nous proposons d'étudier d'une part les fluctuations des populations naturelles de la cératite durant les deux années consécutives 1991 et 1992 dans les biotopes de Mraïssa et de Raf raf de l'étage bioclimatique sub-humide de la Tunisie, très favorables à la pullulation de ce ravageur, le premier étant à fruits d'hiver et le second à fruits d'été ; et d'autre part l'évolution de l'infestation des fruits de *Citrus*dans le biotope de Mraïssa .

I- MATERIEL ET METHODES

I-1- Les biotopes d'étude

Les biotopes de Mraïssa et Raf raf se rapprochent du fait de leur proximité de la mer et s'opposent du point de vue des cultures pratiquées, Mraïssa présente un paysage à monoculture, essentiellement agrumicole ; alors que Raf raf est une zone à polyculture fruitière d'été. Dans ces deux sites, à climat méditerranéen, les précipitations se situent entre 450 et 650 mm par an et se concentrent pendant l'hiver, le maximum est atteint généralement en décembre. La courbe des températures montre un hiver doux. En été (juillet-août), la moyenne maximale quotidienne de la température de l'air dépasse souvent 26 à 28°C, la température du sol peut atteindre jusqu'à 50°C

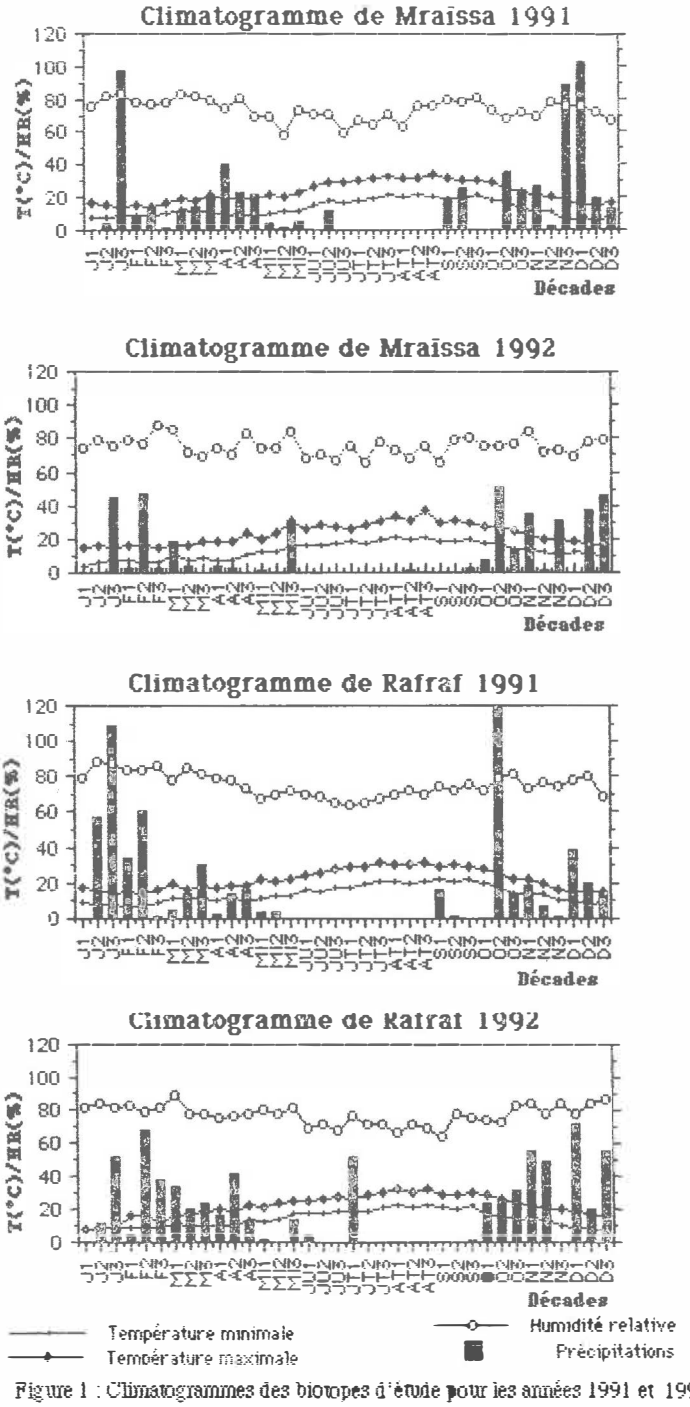


Figure 1 : Climatogrammes des biotopes d'étude pour les années 1991 et 1992.

dans les endroits dégagés. La végétation spontanée est une végétation méditerranéenne à feuilles dures adaptées à ces conditions.

La figure 1 illustre les courbes des moyennes décadaires des températures minimale et maximale, de l'humidité relative moyenne et des précipitations de chacun des deux biotopes d'étude et ce, pour les deux années consécutives d'étude 1991 et 1992 .

Situé en plein cœur du Cap Bon Tunisien, le biotope de Mraïssa est une zone franchement côtière à 36°37' de latitude Nord et à 10°34' de longitude Est ; l'altitude ne dépasse pas le mètre.

Les moyennes, sur 60 ans, des températures extrêmes sont de 10,1°C en décembre et de 26,5°C en août et les précipitations sont de 460 mm. A côté des espèces traditionnellement cultivées telles que la vigne et l'olivier, le paysage agricole se compose essentiellement d'agrumes .

La parcelle expérimentale est un verger d'agrumes situé à 1 km de la mer et couvrant une superficie d'environ 1ha . Il comporte 392 pieds plantés à la densité de 400 pieds /ha (6x4 m), sur un sol sablonneux et comportant un mélange d'espèces et de variétés : clémentinier, oranger Thomson, Valencia late, Double fine, Maltaise et bigaradier .

Ce verger, protégé par des brise-vent, est subdivisé en 9 parcelles limitées chacune par des brise-vent secondaires. Les arbres, moyennement vigoureux, sont âgés d'une quarantaine d'années. Les brise-vent principaux se composent de Tamaris (*Tamarix sp*), les secondaires de Cyprès (*Cupressus spp*) ; des haies de protection sont constituées de figuier de Barbarie et de Lycium, signalons qu'il s'agit de l'espèce *Lycium rubyckarum*, considérée comme étant un hôte secondaire de la cératite (SORIA & CLINE, 1959 ; SORIA & YANA, 1959 ; SORIA, 1962 a ; b).

Quant à la flore de la strate herbacée, elle se compose de plusieurs espèces telles que celles des genres *Calendula, Oloeacus, Chenopodium, Amaranthus, Malva, Cynodon, Lupinus, Chrysanthemum, Astragalus, Rumex, Oxalis, Setaria* etc ...

Tout à fait côtière également, la région de Raf raf se situe à 37°11' de latitude Nord et à 10°09' de longitude Est . L'altitude va en augmentant de la mer vers le village qui offre un paysage de colline atteignant les 100 mètres.

Sur 60 ans, les moyennes des températures extrêmes sont de 11,3°C en décembre et de 27,5°C en août et la moyenne des précipitations est de 653 mm.

Le vent souffle généralement du secteur Est à Est-Nord Est à une vitesse comprise entre 3 et 4 m/s et surtout d'Ouest à Ouest Nord Ouest à une vitesse comprise entre 5 et 6 m / s (moyenne calculée sur 10 ans).

Le biotope offre un paysage à polyculture fruitière surtout estivale, les cultures pratiquées sont essentiellement le figuier commun, le figuier de Barbarie, le pêcher et la vigne ; il importe de souligner que cette dernière espèce n'est pas signalée hôte de la cératite en Tunisie. Trois autres espèces abondent à un degré moindre, il s'agit de l'olivier, l'amandier et les agrumes ; les deux premières espèces ne sont pas non plus signalées pour être des hôtes de la cératite en Tunisie.

Les bordures des pistes renferment du figuier de Barbarie et parfois un autre arbuste de 0,5 à 3 m de haut : *Solanum sodomeum* L. (pomme de Sodome). C'est un arbuste très épineux à poils étoilés, peu rameux qui fleurit de mai à septembre ; feuilles pétiolées ovoïdes de 5 à 13 cm de long ; fruits d'abord marbrés de blanc et de vert devenant des baies d'un jaune éclatant de 2 à 3 cm de diamètre. Cette plante vénéneuse n'est pas signalée hôte de la cératite en Tunisie (SORIA, 1962 a ; b) et nous n'y avons décelé aucune infestation dans les échantillons que nous avons observés durant notre expérimentation.

Afin de travailler dans les conditions naturelles comme l'exige toute étude écologique, les parcelles expérimentales ont été volontairement laissées sans désherbage ni traitement chimique insecticide ou autre.

I-2- Les techniques d'estimation quantitative des populations naturelles de la cératite

I-2-1- Estimation des populations imaginales.

Le suivi des fluctuations de la population naturelle d'adultes de la cératite est assuré par un même système de piégeage installé dans chacun des biotopes de Mraïssa et de Raf raf. Il s'agit d'un ensemble de 5 pièges Rebell jugé plus performant parmi d'autres pièges, appâtés au trimedlure (KATSOYANNOS, 1982 ; QUILICI *et al*., 1987 a ; b ; DELRIO *et al*., 1986, ZERVAS, 1986 ; DELRIO & ORTU, 1988 ; DHOUIBI & GAHBICHE, 1990) ; les pièges sont installés au milieu de la parcelle expérimentale de chaque biotope, disposés en quinconce (BENFATTO & LONGO, 1982, DRUMMOND *et al*., 1984) , distants l'un de l'autre de 12 à 18 m selon les possiblités offertes par les distances de plantation des arbres hôtes (QUILICI *et al*., 1987 ; HENDRICHS *et al*., 1989 ; DELRIO & ZEMREOGLU, 1982) . Le piège est suspendu sur l'arbre hôte à 1m de hauteur dans le côté Sud Est de la frondaison. La capsule à trimedlure (Agrisens TM) est renouvelée une fois toutes les six semaines et la glu une fois tous les 15 à 21 jours. Les dénombrements des adultes capturés se font selon une périodicité hebdomadaire ; du fait de la grande variabilité, la moyenne des cinq pièges est considérée dans le suivi des populations imaginales, qui a duré deux ans dans le biotope de Mraïssa et un an et demi dans le biotope de Raf raf.

I-2-2- Estimation des populations pré-imaginales.

Elle est obtenue au moyen d'examens hebdomadaires d'échantillons de fruits, l'échantillonnage devient plus rapproché (deux fois par semaine) au moment de l'activité de l'insecte. Elle a concerné toutes les espèces de *Citrus* présentes à Mraïssa qui se succèdent en maturité et ce, sur une durée de 9 mois (d'octobre à juin).

La méthodologie d'échantillonnage consiste à choisir au hasard dans chaque parcelle 10 à 15 arbres de façon à avoir une charge totale d'au moins 5000 fruits. Ces pieds sont marqués et servent au prélèvement d'un nombre nécessaire de fruits de façon à disposer d'un minimum de 100 individus pré-imaginaux.

Le suivi de la fluctuation des populations des œufs et des larves dans les fruits est assuré par un échantillonnage systématique des fruits et le dénombrement de tous les stades présents aussi bien morts que vivants.

I-3- Etude de l'infestation des fruits

5 à 10 pieds de *Citrus* ayant une charge totale de 500 à 3000 fruits, selon les variétés, sont choisis pour l'étude de l'évolution temporelle de l'infestation. Les fruits piqués sont comptés et marqués selon deux catégories à savoir les "fruits fraîchement piqués" et les "fruits anciennement piqués" pour déterminer le nombre de fruits infestés. Le suivi a concerné les différentes espèces et variétés de *Citrus* de Mraïssa, c'est à dire successivement par ordre de maturité : les clémentines, les oranges Thomson, Maltaise, Double Fine et Valencia Late.

II- RESULTATS ET DISCUSSION.

II-1- Fluctuations des populations imaginales .

Les données de captures en fonction du temps illustrent l'évolution temporelle de la population imaginale de la cératite dans chacun des biotopes de Mraïssa et de Raf raf. Les courbes de vol (figures 2 et 3) sont interprétées à la lumière des climatogrammes des deux biotopes relatifs à la période d'observation (figure 1).

i) Activité de vol de la cératite à Mraïssa

Durant les deux années 1991 et 1992 , l'adulte de la cératite ne s'est pratiquement pas manifesté depuis le 1er janvier jusqu'au 15 juin à l'exception d'un petit pic d'une moyenne de 2,6 adultes par piège et par semaine apparu à la mi-Janvier pour l'année 92, qui représenterait le reliquat de la population résiduelle devant être émergés du sol.

Vraisemblablement, cette période s'étalant du 1er janvier au 15 juin et durant laquelle nous n'avons pas révelé la présence de l'adulte serait la période d'hibernation de la cératite sous la forme certainement soit de pupe dans le sol soit de larves dans les fruits.

A partir du 15 juin, les effectifs des adultes commencent à augmenter pour atteindre un premier pic le 28 juin pour l'année 91 (avec une moyenne de capture de 35,2 adultes par piège et par semaine) et le 18 juin pour l'année 92 (avec une moyenne de capture de 36,4) (figure 2) . Ce retard de 10 jours enregistré durant l'année 91 serait imputable aux températures enregistrées, légèrement plus basses que celles de l'année 92. La moyenne décadaire des températures minimales de la première décade du mois de juin était de 15,3°C pour l'année 91 et de 16,3°C pour l'année 92 (figure 1) .

Ce premier pic indique un accroissement de la population qui résulterait soit de l'émergence des adultes issus des pupes présentes dans le sol et/ou d'éventuelles arrivées d'adultes de l'extérieur. A partir de ce premier pic indiquant la première génération qui serait développée sur les oranges Valencia late, les courbes de vol de la cératite pour les années 91 et 92 présentent plusieurs autres sommets révélant les générations qui se succèdent et parfois, se chevauchent :

- Pour l'année 1991, le deuxième pic indiquant la deuxième génération a lieu le 23 juillet avec 10 adultes par piège et par semaine (figure 2) . Le troisième pic survient le 16 août où la population atteint une moyenne de 20,4 adultes par piège et par semaine.

Ensuite, la population se maintient faible avec des moyennes de captures oscillant entre 4 et 6 adultes par piège et par semaine jusqu'au 10 octobre où la population atteint son maximum pour toute l'année, soit 80, 4adultes par piège et par semaine. Cette augmentation rapide du niveau de la population qui s'est multiplié pratiquement par 20 est le résultat des conditions climatiques favorables (température moyenne de 23 °C et H.R. de 73% durant la première décade d'octobre) et du début de la réceptivité des fruits de *Citrus* notamment des clémentines. Le pic du 10 octobre correspond donc à la quatrième génération qui s'est développée sur les clémentines et probablement sur les oranges Thomson qui commencent, elles aussi, à être réceptives.

Trois autres pics sont enregistrés successivement les 8 et 28 novembre et le 18 décembre avec des moyennes respectives de 63,8 ; 21,2 et 8,4 mâles par piège et par semaine. Au cours de cette phase automnale, on peut constater que le développement du ravageur est devenu plus rapide. En effet, 3 semaines séparent les 3 derniers pics des mois de novembre et de décembre, 4 semaines séparent celui du 10 octobre et du 8 novembre alors que 8 semaines séparent les pics du 16 août (20,4 adultes /piège/semaine) et du 10 octobre (80,4) entr'eux. Cette durée est légèrement raccourcie en été puisque 7 semaines séparent le pic du 28 juin (35,2) de celui du 16 août (20,4) .

- Pour l'année 1992, après le premier pic du 18 juin avec une moyenne de capture de 36,4 adlutes par piège et par semaine (figure 2) , la population s'est maintenue sensiblement constante durant un mois c'est à dire jusqu'au 16 juillet où elle a alors baissé jusqu'à 16,2 adultes par piège et par semaine. Cette génération qui s'est allongée sur un mois s'est développée sur les oranges Valencia late. Une recrudescence a repris au bout de 3 semaines faisant doubler le niveau de la population le 6 Août (avec une moyenne de capture de 78,6 adultes par piège et par semaine). Le niveau de la population a de nouveau baissé pour accuser une troisième recrudescence très importante doublant encore une fois le niveau de la population à 157,6 adultes par piège et par semaine le 17 septembre, date à partir de laquelle quatre autres générations chevauchantes se poursuivent , c'est à dire les quatrième, cinquième, sixième et septième générations avec des maxima respectifs de 193,2 ; 2084,8 ; 248,6 et 319,4 adultes par piège et par semaine (figure 2) . Il est judicieux de remarquer que la recrudescence qui a noté l'apparition de la cinquième génération est spectaculaire puisque le niveau de la population s'est multiplié par plus de 10 fois par rapport à celui de la génération précédente.

A l'instar de l'année 1991, le développement de la cératite, durant l'année 1992, est devenu plus rapide en automne c'est à dire en septembre, octobre, novembre et même en décembre, mois durant lequel les températures enregistrées ont été exceptionnelles puisque le minimum enregistré est de 15,2°C alors que la moyenne des températures du mois de décembre sur 60 ans est de 10,1°C, on retrouve cette température deux fois durant les 8 années précédentes (tableau 1) .

Tableau 1 : Moyennes des températures mensuelles du mois de décembre des 8 dernières années à Mraïssa .

Année	1984	1985	1986	1987	1988	1989	1990	1991	1992
Température (°C)	12,9	14,3	12,3	15,0	13,0	15,5	11,9	11,9	15,2

En conclusion, l'étude des fluctuations de la population de la cératite dans le biotope de Mraïssa durant les deux années 1991 et 1992 effectuée sur les Citrus, a révelé le développement de 7 générations annuelles pour chacune des deux années d'étude (figure 2) . En 1991 , le niveau maximal de la population est relativement bas et les 3 générations automnales sont chevauchantes alors qu'en 92, le niveau maximal atteint est 25 fois plus important que celui de l'année précédente et l'ensemble de 7 générations sont chevauchantes. La durée du développement post-embryonnaire est longue en hiver et relativement courte au printemps et en automne (TASSAN *et al*, 1982) .

ii) Activité de vol de la cératite à Raf raf.

Dans le biotope de Raf raf , l'activité de vol de la cératite a été suivie durant un an et demi puisque le réseau de piégeage n'a été installé qu'au début du mois de juillet 1991 pour se poursuivre jusqu'à la fin du mois de décembre 1992 (figure 3) .

- La courbe de vol de la deuxième moitié de l'année 91 montre que la cératite évolue durant ces 6 mois en 4 générations indiquées par 4 pics survenus successivement le 23 juillet ; le 27 août, le 1er et le 19 octobre avec des effectifs respectifs de 509,8 ; 236 ; 285,8 et 30,2 adultes par piège et par semaine (figure 3) . Il faut remarquer que le 2ème pic est assez étalé et peut correspondre au développement de deux générations chevauchantes favorisées par les températures élevées de l'été (août-septembre) et l'importance maximale des fruits hôtes dans ce biotope (figues, pêches, pommes et figues de Barbarie).

D'autre part, le dernier pic (19 novembre) qui indique un niveau de population 9,4 fois plus bas que le précédent, se trouve à une distance 1,4 fois supérieure aux deux distances séparant les 3 autres pics, ceci prouve que le développement du ravageur est devenu plus lent avec le début des températures basses de l'hiver, cette génération serait développée sur les figues de Barbarie, seuls fruits hôtes disponibles pendant cette période de l'année.

- Durant l'année 1992, et comme dans le biotope de Mraïssa, les captures sont nulles de janvier jusqu'à la mi-Juin, date à laquelle le niveau de la population commence à s'élever pour atteindre un premier maximum le 27 juillet avec une moyenne de capture de 135,8 adultes par piège et par semaine (figure 3) . Cette augmentation rapide et très importante de la population, serait dûe manifestement à l'éclosion des pupes présentes dans le sol et aux éventuelles migrations des adultes à la recherche de l'hôte, lesquelles éclosions et migrations sont favorisées, d'une part par les températures de l'été très favorables au développement de la cératite et d'autre part par la maturité

d'une grande gamme de fruits (prunes, abricots, figues). En effet, cette première génération serait développée dans les abricots qui comportaient des asticots de cératite depuis le 2 juin 1992.

Le deuxième pic du 14 septembre correspondant à 525,2 adultes par piège et par semaine, chevauche avec celui de la première génération avec un niveau de population 3,8 fois plus important. Ce pic qui s'étale sur 8 semaines avec un effectif élevé, indique que deux générations cumulées et confondues en une seule seraient développées et ce, grâce à des conditions climatiques très favorables et à la maturité d'autres plantes hôtes (pêches et figues de Barbarie) qui viennent s'ajouter aux premières (prunes, abricots et figues).

Les trois autres pics de la courbe de vol durant l'année 92 surviennent successivement le 31 octobre, le 13 novembre et le 4 décembre avec des effectifs respectifs de 249,5 ; 168,5 et 165 adultes par piège et par semaine, ces trois pics marquent trois autres générations chevauchantes (figure 3) .

Un fait important se remarque au sujet de la dernière génération, c'est que, contrairement à l'année 1991 durant laquelle cette dernière génération, survenue le 19 novembre, a accusé une baisse du niveau de la population (de 9,4 fois par rapport au niveau précédent) ; durant l'année 1992, cette même génération a enregistré un niveau de population presqu'équivalent au précédent, elle a accusé en plus un retard puisque son apparition n'a eu lieu que le 4 décembre. Ce phénomène serait imputable aux conditions climatiques relativement clémentes enregistrées durant l'automne 92, durant lequel les températures se sont maintenues assez élevées jusqu'au mois de décembre. En effet, la température mensuelle moyenne enregistrée durant le mois de décembre 92 est de 12,3°C alors que sa température mensuelle moyenne calculée sur 60 ans est de 11,3 °C à Raf raf , ces températures assez élevées, qui ont fait augmenter cette moyenne de 1°C, ont également une moyenne supérieure à celles des années précédentes. Le tableau 2 montre, à titre indicatif , les moyennes mensuelles du mois de décembre des 8 années précédentes et celle de 92.

Tableau 2 : Moyennes des températures mensuelles du mois de décembre à Raf raf des 8 dernières années .

Année	1984	1985	1986	1987	1988	1989	1990	1991	1992
Température (°C)	11,9	12,6	11,4	13,4	12,0	14,7	11,0	11,2	12,2

Cette élévation inhabituelle de la température durant le mois de décembre 1992 a donc permis à la cératite de pulluler davantage durant la dernière génération.

En définitive, dans le biotope de Raf raf à polyculture fruitière estivale, la cératite a évolué en 4 générations durant la deuxième moitié de l'année 91 et en 6 générations, chevauchantes 3 par 3, durant toute l'année 92 avec des niveaux de population très élevés. Pour 1991, seule la dernière génération a un niveau plus bas.

iii) Conclusion.

L'étude des fluctuations des populations imaginales de la cératite dans deux biotopes opposés du point de vue hôtes (polyculture estivale et monoculture hivernale) et à conditions climatiques similaires (étage bioclimatique sub-humide de la Tunisie) a montré que le ravageur a développé:

- 7 générations à Mraïssa aussi bien durant l'année 91 que durant l'année 92,
- 6 générations, chevauchantes trois par trois, durant l'année 92 à Rafraf et
- 4 générations de juillet à décembre 1991 à Raf raf.

Par ailleurs, les générations les plus courtes sont celles de l'automne alors que les plus longues sont celles de l'hiver. Ce résultat rappelle celui de TASSAN *et al.* (1982) qui trouvent que la durée des différentes générations de la cératite, en Californie, est variable d'une saison à l'autre.

II-2- Fluctuations des populations pré-imaginales.

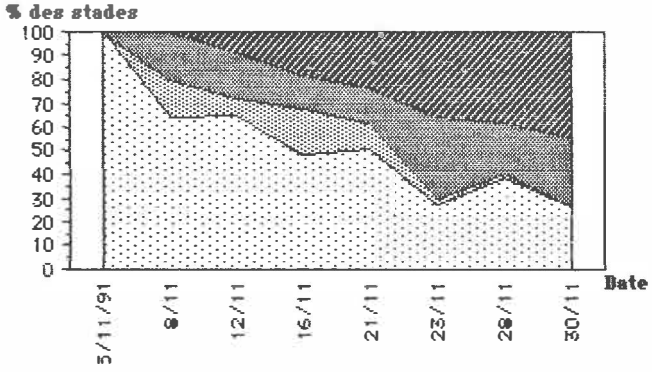
Le suivi des fluctuations des populations pré-imaginales a été effectué seulement dans le biotope de Mraïssa sur une durée de neuf mois (de novembre 1991 à juin 1992) et ce, pour toutes les plantes hôtes présentes dans ce biotope c'est à dire, successivement par ordre chronologique de maturité : les clémentines, les oranges Thomson, les oranges Maltaise, les oranges Double fine et les oranges Valencia late (figure 4) . La proportion d'un stade évolutif aussi bien vivant que mort est déterminée par rapport au total des individus pré-imaginaux qui composent la population.

i) Evolution temporelle des pontes.

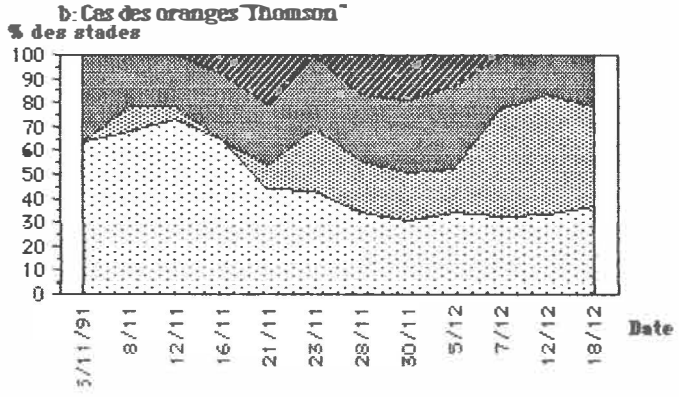
- Dans les clémentines, l'échantillonnage effectué avant le 5 Novembre n'a révélé la présence d'aucun individu pré-imaginal quoi que les fruits présentaient quelques piqûres stériles et sur lesquelles nous reviendrons. Les pontes ont donc commencé le 5 novembre ; le premier pic est apparu le 12 novembre un deuxième le 21 novembre et un troisième le 28 novembre (figure 4 a).

Le pic maximal du 12 novembre est survenu 3 jours après la capture massive des adultes de la 5ème génération (63 adultes par piège et par semaine). Les pontes chutent alors au profit des jeunes stades larvaires qui se développent, le niveau d'oviposition reste relativement constant et chute avec la fin de la 5ème génération (déclin du niveau de la population de 63 à 10,8 adultes par piège et par semaine).

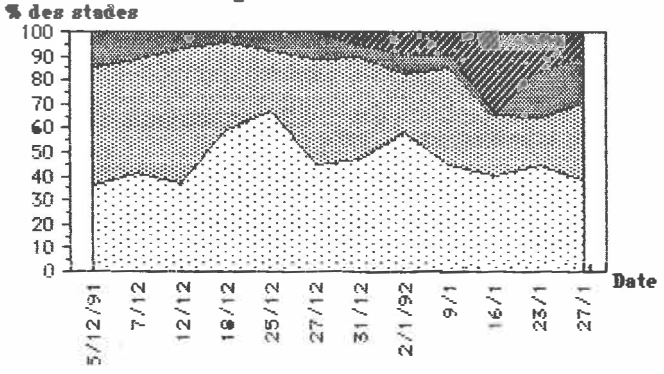
a: Cas des clémentines



b: Cas des oranges "Thomson"



c: Cas des oranges "Maltaise"



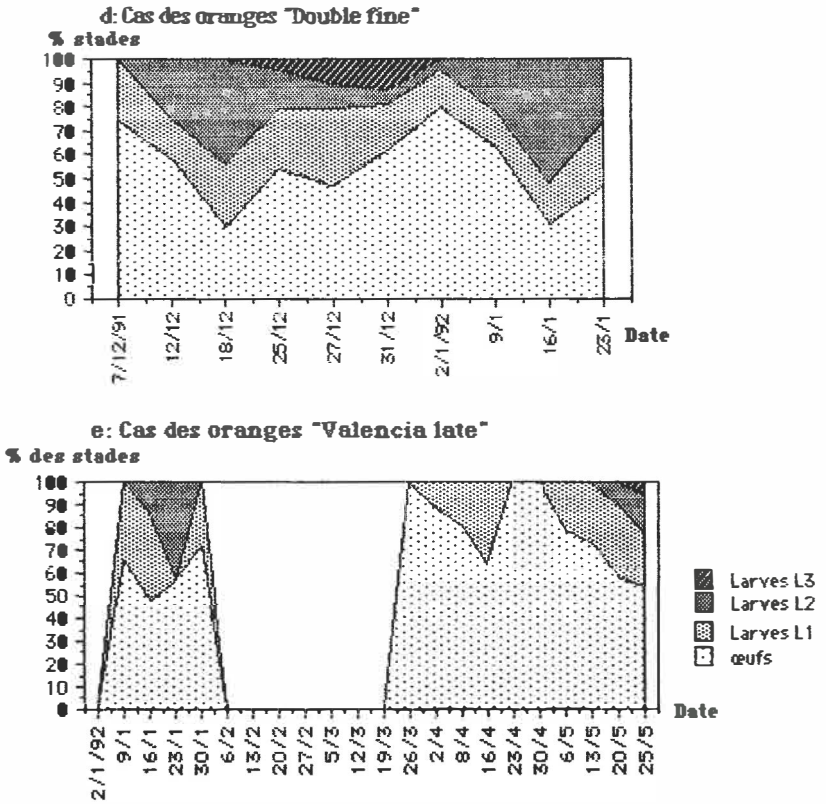


Figure 4 : Evolution temporelle de la population pré-imaginale dans les fruits de Citrus.

La dernière génération (pic du 28 Novembre de la population imaginale) représentée par une proportion de 39 % d'œufs, résulte de la ponte des femelles qui auraient apparu à cette même date (21,2 adultes par piège et par semaine).

- Dans les oranges Thomson, nous retrouvons un niveau d'oviposition qui se maintient constant jusqu'au 16 novembre (figure 4 b) , date à partir de laquelle il chute contrairement aux clémentines où il demeurait constant ; ceci se rattacherait au fait qu'à cette période-là , la cératite préfère les clémentines pour pondre plutôt que les oranges Thomson. D'autre part, le passage de l'hôte "orange Thomson" à l'hôte "clémentine" se fait sentir le 30 novembre ; date à laquelle nous avons noté une population pré-imaginale plus importante sur Thomson .

- Dans les oranges "Maltaise", la cératite enchaîne ses générations, un premier pic d'oviposition survient le 25 décembre et résulterait des pontes des femelles apparues le 18 décembre (pic du 18 décembre de la population imaginale), le niveau de ce premier pic est relativement bas, corrélé avec le niveau de la population imaginale également assez bas (8,4 adultes par piège et par

semaine). Ensuite, le niveau d'oviposition baisse jusqu'au 27 décembre favorisé sans doute par le déclin des populations imaginales et par les basses températures qui inhibent la ponte (température minimale de 5,4 °C le 27 décembre 91 à Mraïssa) (figure 4 c) .

Les pontes reprennent lentement jusqu'au 2 janvier, date à laquelle, elles enregistrent un second pic de 58,5% ce pic d'oviposition, auquel ne correspond aucun accroissement de la population imaginale, serait dû au faible effectif des stades de l'échantillon, ou à la limite, il serait celui des femelles issues de la population résiduelle de la génération du 18 décembre et il serait favorisé par une température clémente. En effet, le 2 Janvier 92 qui était le jour le plus chaud de ce mois, la température minimale enregistrée était de 10,8°C. A partir du 9 janvier, le niveau reste constant jusqu'au 27 janvier (date de la récolte) .

- Dans les oranges "Doube fine" , la situation est presque identique à celle des oranges "Maltaise" puisque ces deux hôtes se chevauchent dans leur maturité puis se succèdent. Cependant, il est important de remarquer que les pontes chutent considérablement le 18 décembre dans les "Double fine" du fait que la cératite préfère les oranges "Maltaise" à ce moment là ; en revanche elles augmentent beaucoup plus le 2 Janvier (figure 4 d) , indiquant que les oranges "Double fine" deviennent plus réceptives que les "Maltaise" .

Toutefois, les oranges "Maltaise" permettent mieux le développement des œufs que les "Double fine" du fait que le déclin de l'effectif des œufs du 16 janvier, relatif à la fin de la dernière génération, est accentué dans les oranges "Double fine" alors qu'il est presque constant dans les oranges "Maltaise" .

- Les oranges "Valencia late" ont commencé à reçevoir les œufs de la cératite le 9 janvier 92, la population des pré-imaginaux s'est annulée à partir du 6 février c'est à dire à la manière de la population imaginale. Au printemps , les pontes ont repris le 19 mars (figure 4 e) , favorisées par l'avènement des températures clémentes et par le début de la réceptivité des "Valencia late" ; ces pontes qui apparaissent en l'absence de captures d'adultes dans les pièges, seraient l'œuvre des premières femelles qui auraient migré à partir des bigarades présentes dans ce biotope.

Ensuite, les pontes chutent jusqu'au 16 avril à cause des basses températures et reprennent ensuite rapidement avec l'élevation de la température atteignant un pic qui s'étale sur la semaine du 23 au 30 avril.

Enfin, durant tout le mois de mai, on n'enregistre plus de ponte du fait de l'absence des adultes et des températures relativement fraîches enregistrées. Ceci explique d'ailleurs le faible taux de piqûre obtenu à la récolte .

ii) Evolution temporelle des larves.

Le développement larvaire suit généralement les ovipositions (figure 4) ; il faut cependant remarquer que l'effectif des jeunes stades (L_1 et L_2) l'emporte sur celui du stade larve âgée (L_3), ceci indique qu'il n'y a pas de développement du 2ème stade en 3ème du fait de la mortalité enregistrée dans les écorces

En conclusion, l'étude des fluctuations des populations naturelles de la cératite dans les biotopes de Mraïssa et Raf raf, respectivement sur les agrumes et sur les fruits d'été, a montré que la cératite développe 7 générations annuelles à Mraïssa et 6 générations, chevauchantes 3 par 3, à Raf raf. Ce résultat se rapproche de celui de BENFATTO & LONGO (1982) obtenu en Sicile.

L'étude a en outre mis en évidence l'absence, ou du moins la rareté, des adultes de cératite durant l'hiver et le printemps (du 1er janvier jusqu'au 15 juin). Nos résultats sont en contradiction avec ceux de CHEICKH *et al.* (1972) qui pensent que l'adulte est présent toute l'année ; ils concordent, en revanche, avec ceux de BENFATTO & LONGO (1982) et de ORTU & PROTA (1988) qui obtiennent des captures nulles sur *Citrus* de janvier à juin respectivement aux Iles de Sicile et de Sardaigne.

En somme, la période de développement et de reproduction de la cératite sur les fruits d'été et sur les agrumes s'étale du début du mois de juin jusqu'à la fin du mois de décembre, il y a donc une interruption en hiver.

Par ailleurs, le suivi de la population pré-imaginale dans les fruits de *Citrus* a montré une concordance avec les captures des adultes dans le biotope de Mraïssa ; ce résultat est en accord avec celui de M'AAREF (1989) qui note une synchronisation entre les captures et l'évolution des œufs et des larves dans les agrumes (fruits d'*Agrumes sinensis*).

II-3- Etude de l'infestation des fruits

L'évolution des infestations des différentes espèces hôtes est représentée sur le même graphique afin de mettre en évidence la succession des infestations au rythme de la succession de la réceptivité et de la maturité des fruits hôtes d'une part, d'autre part, l'importance relative du niveau d'infestation des différentes plantes hôtes.

Les infestations commencent timidement en automne (24 octobre) sur les clémentines une première augmentation de l'infestation se fait sentir le 8 novembre puisqu'elle passe de 1,7% (5 novembre) à 2,5% (8 novembre) (figure 5). Elle se stabilise quelque peu pendant une semaine, jusqu'au 16 novembre, pour augmenter à une allure plus vertigineuse de jour en jour jusqu'au 5 décembre, date de la récolte des clémentines. Entre temps, les infestations ont déjà commencé sur les oranges Thomson, le premier contrôle effectué le 5 novembre révèle 2,45% d'infestation, ce taux s'élève rapidement le 8 novembre à 6,62% et ce, à la manière de l'infestation des clémentines. Cette augmentation brusque de l'infestation est le résultat de la recrudescence de la population imaginale du 8 novembre qui atteint 63 adultes/ piège/semaine (figure 2).

Les infestations dans les oranges "Thomson" se poursuivent à un rythme assez régulier avec un niveau relativement élevé atteignant 42,95% au dernier contrôle.

Après la récolte des clémentines et des oranges Thomson, ce sont les fruits du groupe des sanguines (Maltaise et Double fine) qui arrivent à maturité et deviennent réceptifs, offrant les possibilités de ponte à la cératite ; les infestations de ces deux hôtes chevauchent cependant avec celles des oranges "Thomson" sur deux semaines (du 5 au 19 décembre).

Les oranges "Maltaise" et "Double fine" rencontrent un niveau de population imaginale relativement bas (figure 2) , permettant juste de maintenir le niveau d'infestation initial avec une légère augmentation de l'infestation laissant le niveau général relativement constant. Il faut remarquer la présence également d'une augmentation de l'infestation des oranges Maltaise enregistrée le 23 janvier et qui serait dûe à la récolte tardive des Maltaises donnant plus de temps et plus de chance au développement larvaire et aux nouvelles piqûres (figure 5) .

Enfin, les oranges "Valencia late", qui mûrissent à partir de la deuxième quinzaine du mois de mai, rencontrent des conditions climatiques favorables notamment de température mais sans reprise d'activité de vol du ravageur (à cause des captures nulles de janvier à juin). Le niveau d'infestation de cette dernière espèce hôte s'est maintenu très bas depuis les premières piqûres de Janvier jusqu'à la première quinzaine du mois de mai où les infestations ont augmenté grâce à l'émergence et à la migration des premières femelles (figure 5) . Toutefois, les oranges Valencia late sont arrivées à maturité avec un niveau d'infestation très bas (2,9%) et ont donc échappé aux attaques de la cératite d'autant plus que la récolte a été très précoce (le 25 mai) .

CONCLUSION

L'étude de l'évolution temporelle de l'infestation des fruits de *Citrus* dans le biotope de Mraïssa a montré :

- Une succession des infestations au rythme de la succession de maturité des fruits hôtes.
- Un niveau d'infestation variant en fonction des plantes hôtes : les oranges Maltaise sont apparemment les plus touchées alors que les oranges Valencia late ont échappé à l'attaque(Figure 5).
- Le niveau d'infestation des différentes plantes hôtes (figure 5) est le résultat d'une augmentation du niveau de capture de la population imaginale dans les pièges (figure 2) . Il s'agit donc d'une relation de "cause à effet" qui existe entre les deux variables aléatoires "infestations" et "captures" qui représentent l'une et l'autre un facteur non contrôlé. Nos résultats rappellent ceux de DELANOUE ,1957, DELANOUE & SORIA (1962 a & b) qui observent une augmentation du taux de piqûre conséquente à une augmentation du taux de capture sur *Citrus* et de celui de M'AAREF (1989) qui note une évolution synchrone entre le degré de maturité des oranges et le pourcentage de fruits attaqués par la cératite.Ce dernier semble être plus important du coté Sud de la plante-hôte (DHOUIBI *et al.* 1993 et DHOUIBI & GAHBICHE 1993).

En ce qui concerne la dynamique des populations du ravageur, le suivi des fluctuations des populations imaginales et pré-imaginales nous a permis de noter que la cératite accomplit 7 générations à Mraïssa et 6 générations à Raf raf, chevauchantes 3 par 3. Ce résultat concorde avec les observations de BENFATTO & LONGO (1982) faites en Sicile.

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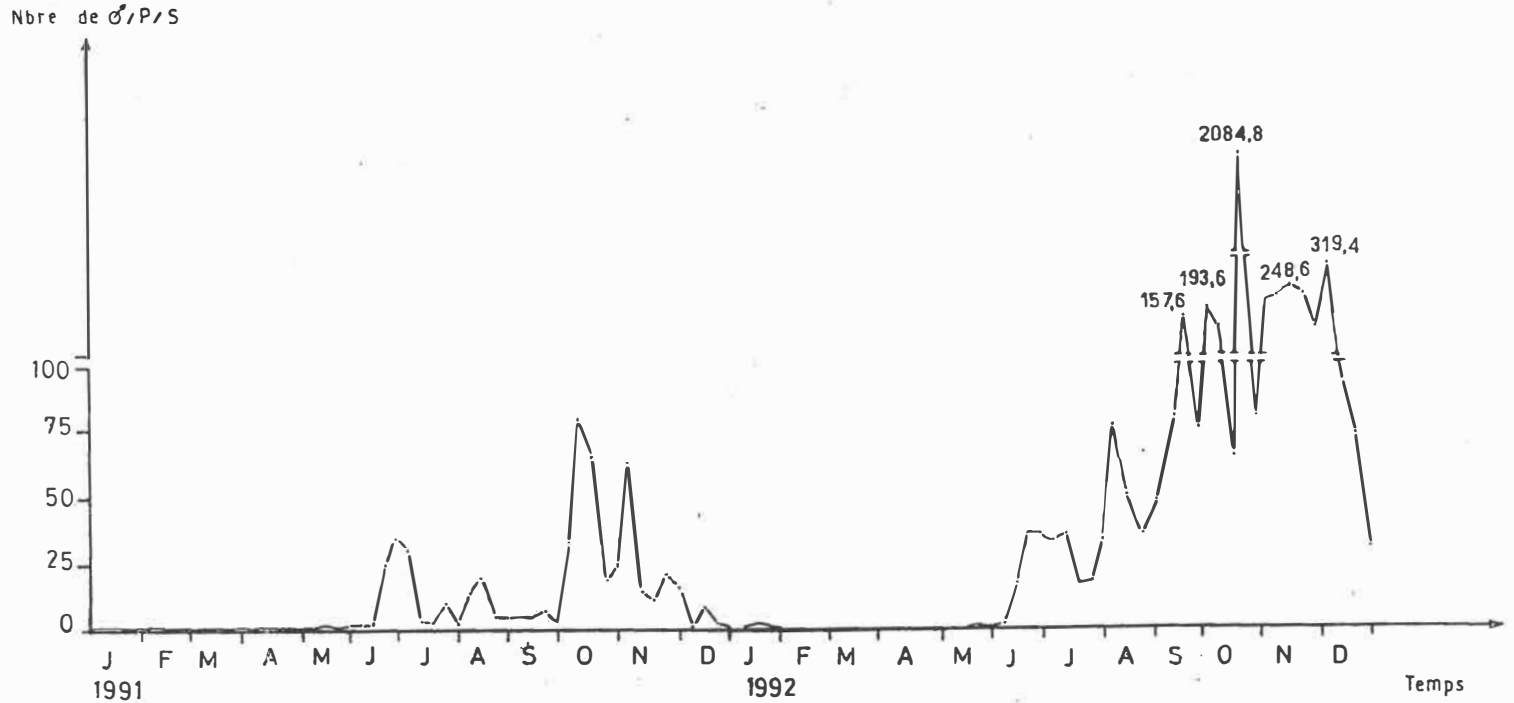

Fig: 2 Courbe de vol de la cératite dans le biotope de Mraïssa

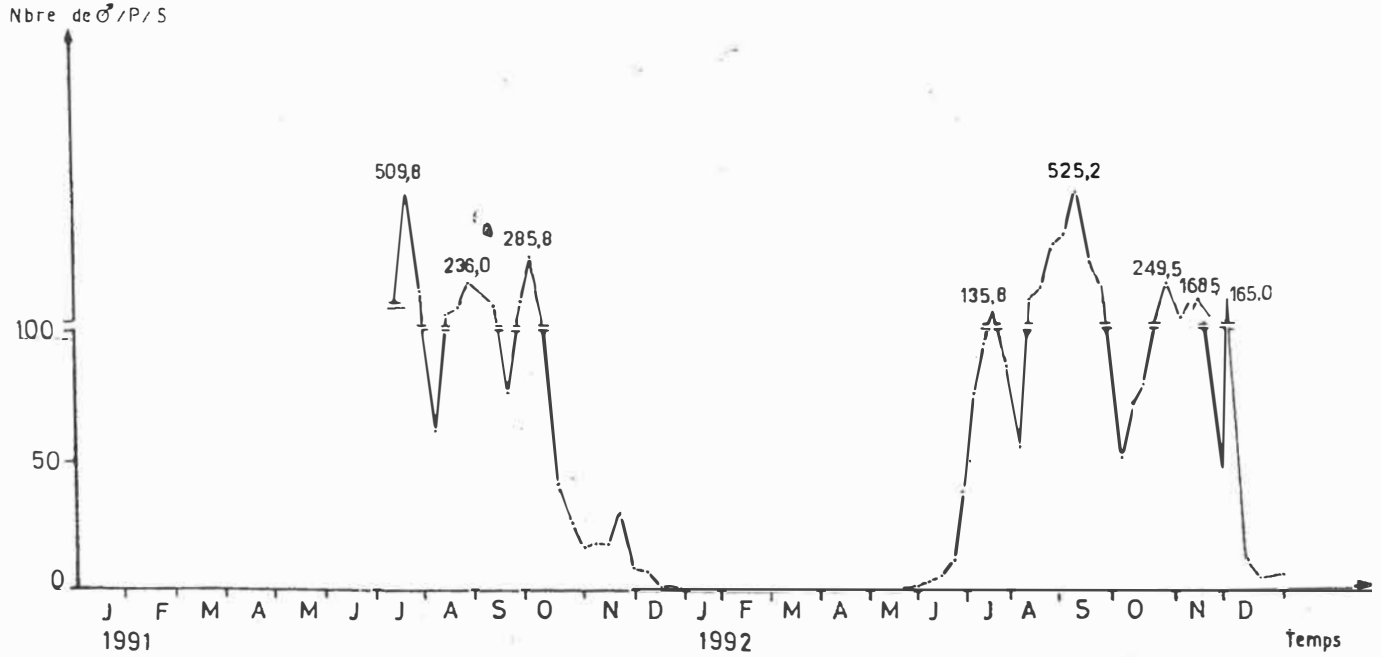

Fig : 3 Courbe de vol de la cératite dans le biotope de Rafraf

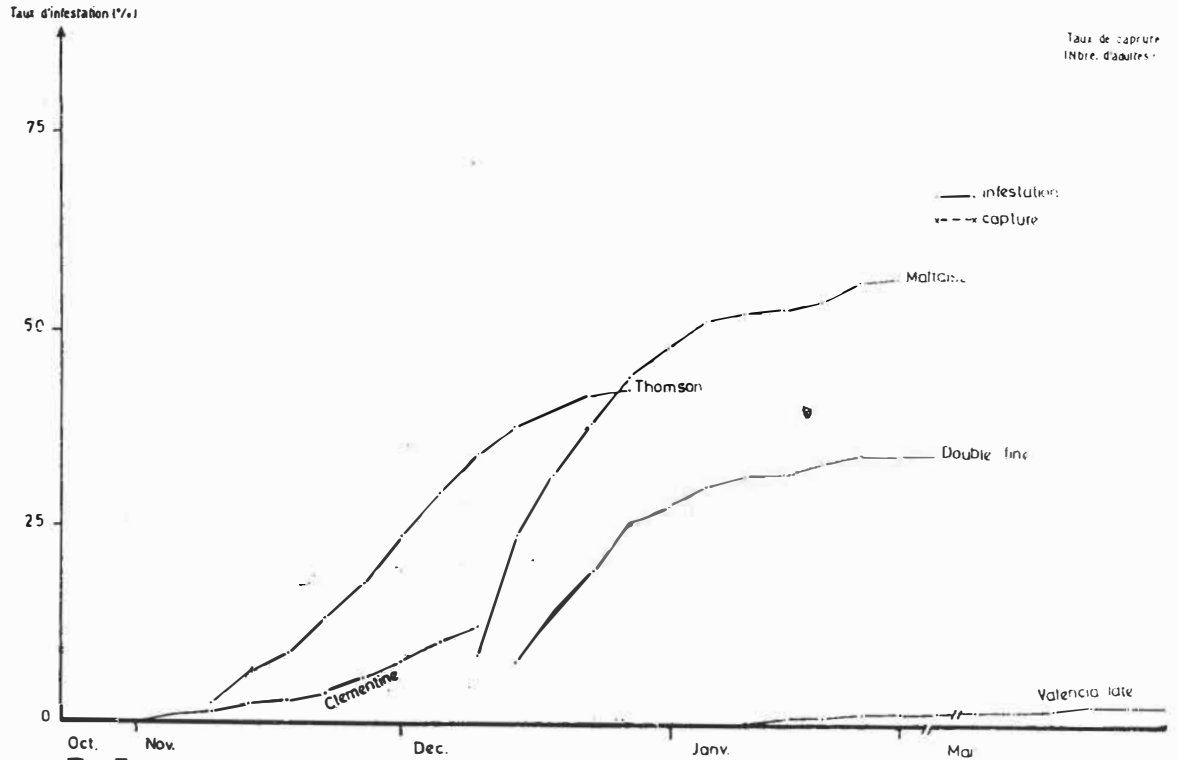

Fig 5 Evolution temporelle de l'infestation de 5 espèces de Citrus étudiée dans le biotope de Mraisso

PROBLEMÁTICA DA MOSCA DO MEDITERRÂNEO *CERATITIS CAPITATA* (WIED.) NOS CITRINOS EM PORTUGAL

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Abstract

The impact of Mediterranean fruit fly *Ceratitis capitata* (Wied.) in citrus groves in Portugal. *Ceratitis capitata* (Wied.) is considered a key pest in citrus groves in Portugal, specially in Algarve, in spite of the fact that oranges do not provide the optimal conditions to its development. Total mortality ratio of eggs and larvae is about 97%.

The biology of this species (high biological potential, adult's longevity, their mobility and polyphagia), favorable climatic conditions, farming systems and deficient and irregular phytosanitary practices, all contribute to give the Mediterranean fruit fly a pest status.

That is why in Algarve (the major citrus production region of Portugal) we can find adults throughout the year. But the major fruits loss occurs during the Summer and beginning of Autumn, when *C. capitata* adult population has its peaks.

Resumo

Apesar dos frutos dos citrinos não oferecerem condições que favoreçam o desenvolvimento de *Ceratitis capitata* (Wied.), provocando nos ovos e larvas uma mortalidade que, no conjunto, ultrapassa em geral uma percentagem de 97%, mesmo assim esta espécie constitui uma praga chave para os citrinos em Portugal, principalmente no Algarve.

Contribuem para que a mosca do Mediterrâneo tenha este estatuto, a biologia da espécie (elevado potencial biótico , longevidade dos adultos, mobilidade destes e polifagia), as condições climáticas que lhe são favoráveis, assim como a estrutura fundiária da propriedade, e, ainda, a deficiente e irregular prática fitossanitária.

Esta situação, permite que no Algarve (a principal região produtora de citrinos em Portugal) possam ser encontrados adultos em todos os meses do ano. Mas, a maior gravidade dos ataque verifica-se durante os meses de Verão e princípio do Outono, período em que a abundância populacional dos adultos de *C. capitata* atinge os valores mais elevados.

1-Introdução

Para quem conheça a elevada polifagia e a estreita dependência de *Ceratitis capitata* (Wied.) do condicionalismo climático, bastaria um simples relance sobre os valores médios

mensais da temperatura e da humidade relativa em Portugal, para ter uma primeira noção da importância desta espécie, não só no continente, como também (e por razões ainda mais fortes) nas regiões insulares.

No que se refere a Portugal continental, embora com todas as interrogações resultantes das particularidades microclimáticas regionais e das características dos hospedeiros, não se pode deixar de admitir que, perante a orientação norte-sul do território, será nas regiões do centro e, principalmente do sul, que *C. capitata* encontra as melhores condições climáticas para proliferar e constituir uma praga com elevada importância económica em relação a diversas espécies de fruteiras.

Referenciada por MaCleay em 1829 para os Açores e Madeira (Back & Pemberton, 1915), por Pereira Coutinho em 1898 e por Verissimo de Almeida (1900) para o continente português, *C. capitata* manifesta-se como praga importante para as culturas de pessegueiro, damasqueiro e citrinos, embora revele elevada apetência e êxito biológico nos frutos de outras plantas cultivadas, com destaque para o caso da figueira. Mas, conforme a importância das culturas e as características dos frutos que são atacados, no que respeita à sua susceptibilidade aos ataques e à taxa de sobrevivência de *C. capitata*, assim é variável a importância económica que esta praga assume.

A nivel mundial, *C. capitata* constitui uma das espécies de insectos mais estudadas. No espaço português, porém, os trabalhos que lhe têm sido dedicados podem considerar-se escassos e muito distanciados no tempo, apesar da importância que esta espécie tem no cômputo geral das pragas de insectos que afectam as fruteiras.

Da bibliografia portuguesa deve ser destacado o trabalho monográfico de Vieira (1952), realizado na Ilha da Madeira, pela diversidade de temas abordados, sua profundidade e, em muitos aspectos, pela sua actualidade. A este trabalho (que teve na sua origem a realização de um estágio de fim do curso de Engenharia Agronómica), seguiu-se um outro com a mesma finalidade, sobre *C. capitata*, realizado por Entrudo Júnior (1955) em Portugal continental, no Algarve.

Desde meados da década de 1960 a meados da década seguinte, foram feitas criações em massa de *C. capitata* no Departamento de Entomologia, da Estação Agronómica Nacional. Estas criações mantidas pelo investigador Magalhães Silva tiveram como objectivo a obtenção de parasitóides para utilização contra a mosca da azeitona *Dacus oleae* (Gmelin), bem como para o emprego do método dos machos esterilizados contra *C. capitata* na Ilha da Madeira.

O estudo de *C. capitata* foi retomado por Piedade Guerreiro *et al.* (1985), tendo o primeiro autor realizado a sua dissertação para obtenção do Grau de Doutor em Biologia, sob o tema da esterilização deste insecto por meio de radiações ionizantes (Guerreiro, 1987)

Indo ao encontro do avanço que nos últimos anos se tem verificado na luta contra *C. capitata*, consolidado pelas actuais facilidades da criação em massa e concretizado pelos assinaláveis êxitos resultantes da aplicação da técnica dos machos esterilizados, desde 1989 que no Departamento de Entomologia, da Estação Agronómica Nacional, se têm realizado estudos, mas com maior incidência sobre aspectos da bioecologia desta espécie, orientados principalmente no sentido da melhoria dos conhecimentos sobre a dinâmica das populações. A par destes estudos e dos resultados já alcançados (Carvalho & Fernandes, 1993; Carvalho & Pereira, 1993; Pereira & Carvalho, 1993), tem sido dado apoio à

realização de estágios de fim de curso: um finalizado (Baptista, 1990), referente a estudos complementares da luta autocida; dois outros sobre aspectos da dinâmica das populações em citrinos do Algarve (dos quais um deles se encontra concluído e o outro em fase avançada da sua base experimental) e uma dissertação de mestrado em Protecção Integrada abordando idêntica temática (também esta com a base experimental concluída).

Foi determinante para a opção tomada sobre a realização dos estudos principalmente nos citrinos do Algarve, a importância que tanto *C. capitata* como esta cultura têm nesta região e também o interesse de se desenvolverem metodologias respeitantes à monitorização e a estudos sobre dinâmica das populações, visando a aplicação dos conhecimento na luta contra esta praga, a nível local e nacional. A Divisão de Protecção da Produção Vegetal, da Direcção Regional de Agricultura do Algarve, tem dada a sua colaboração na realização destes estudos ,os quais foram inicialmente previstos para possibilitarem também a obtenção de conhecimentos com interesse para a aplicação de estratégias inseridas no âmbito da projectada luta autocida contra *C. capitata* na Ilha da Madeira.

2-Panorâmica geral sobre a distribuição e abundância de *C. capitata*

São numerosos os factores que exercem influência na distribuição e abundância de *C. capitata*. Desses factores, destacam-se as condições climáticas e alimentares pela sua marcada importância e interferência directa e indirecta na biologia da espécie.

2.1-Influência das condições climáticas

De acordo com a classificação de Köppen, o clima de Portugal continental é do tipo Cs (clima mediterrânico), tendo como característica mais importante a existência de uma estação seca no Verão e chuvosa no Inverno. Este tipo de clima, que pela sua definição se prende fundamentalmente com o regime pluviométrico e com a temperatura, abrange apenas uma área de 1,7% da superficie terrestre (Azevedo, 1980). Tal tipo de clima, manifesta-se por particularidades regionais de grande impacto ecológico que não devem ser ignoradas. Dessas salientam-se: a latitude associada à disposição norte-sul do território; a orografia relacionada com a altitude e com a exposição aos ventos dominantes; e, a influência das condições climáticas externas que localmente variam com as distâncias à faixa costeira ou à fronteira interior.

O clima mediterrânico que caracteriza o território não deixa assim de apresentar variações as quais, segundo Azevedo (1980), vão desde o xeromediterrânico no Sul ao submediterrânico no Nordeste e nas zonas montanhosas do Centro, sem exclusão de condições climáticas particulares em pequenas áreas.

Não esquecendo a influência do solo, estas condições climáticas são determinantes da vegetação e exercem simultaneamente uma acção decisiva sobre o potencial biótico dos componentes das respectivas biocenoses, condicionando o número de gerações anuais, a taxa de multiplicação, a abundância sazonal das populações, a distribuição geográfica e a própria importância económica.

É neste contexto que em Portugal continental a entomofauna, em particular *C.*

capitata, se subordina ao determinismo do clima mediterrânico, o qual em manchas de contornos imprecisos e com acentuadas variações regionais e locais é favorável a esta espécie numa extensa área no Centro e, sobretudo, no Sul do País. Os contornos destas manchas dependem da taxa de sobrevivência de *C. capitata* conforme as condições climáticas, principalmente da conjugação da temperatura com a humidade relativa, mas também de todo um conjunto de influências que afectam estes factores, como diferenças de altitude, de exposição aos ventos dominantes e do regime pluviométrico, acompanhadas pela ocorrência de geadas e de outras influências de natureza trófica e ambiental.

Como é bem conhecido, a temperatura é o factor do clima que maior influência directa exerce sobre o desenvolvimento, ritmo da actividade diária e sazonal (com implicações do fotoperíodo), comportamento, sobrevivência, abundância populacional e distribuição geográfica.

É a resultante deste complexo de factores e suas interrelações que, localmente, determina a composição biótica dos ecossistemas, afectando no caso da espécie considerada a sua capacidade de sobrevivência e a abundância populacional. Mas, dadas as variações dos factores referidos e das suas interferências, não é possível delimitar com rigor em relação a *C. capitata* as zonas óptimas, favoráveis, desfavoráveis ou impossíveis definidas por Bodenheimer (1951). Acrescente-se que, para a determinação destas zonas, não devem ser ignorados os efeitos ao longo do ciclo de vida de *C. capitata* dos valores extremos dos principais factores, em especial do clima, com destaque para a temperatura. Não são assim indiferentes para a biologia de *C. capitata* as amplitudes térmicas, pelos efeitos que podem ter sobre a longevidade dos adultos, capacidade de postura, duração do desenvolvimento dos estados imaturos e taxa de sobrevivência.

Ponderadas tais influências sobre a biologia de *C. capitata*, não deixa contudo de ter interesse a análise do possível impacto dos valores médios da temperatura e da humidade relativa sobre a sua distribuição e abundância regional. Para o efeito, servem-nos de base os dados das estações meteorológicas nacionais (Anónimo, 1970) e os limites da temperatura (10ºC) e da humidade relativa (60%), utilizados por Bodenheimer (1951) para estabelecer, através de climatogramas, as zonas favoráveis e desfavoráveis à ocorrência de *C. capitata*.

De um modo geral, em Portugal continental, os valores médios da temperatura e da humidade relativa podem ultrapassar os limites das zonas favoráveis à ocorrência de *C. capitata* nos períodos mais quentes e secos do Verão, quando a humidade relativa é inferior a 60% e principalmente nos períodos, de duração muito variável consoante as regiões, em que os valores médios da temperatura são inferiores a 10ºC.

Nestas condições, podemos fazer uma breve análise da zonagem de *C. capitata* considerando as seguintes quatro classes de ocorrência:

Classe I-regiões desfavoráveis durante um período igual ou superior a 6 meses (Inverno e Verão).
Classe II-regiões desfavoráveis durante um período igual ou superior a 3 meses (Inverno).
Classe III-regiões desfavoráveis entre 1 a 2 meses.
Classe IV-regiões favoráveis durante todo o ano.

Saliente-se que as condições desfavoráveis nos meses de Inverno e Verão se referem, respectivamente, ás baixas temperaturas, e ao tempo seco associado à combinação dos

valores de temperatura e humidade relativa.

De acordo com este critério, *C. capitata* situar-se-á na Classe I numa área que abrange desde e extremo norte de Trás-os-Montes numa extensa faixa interior até ás proximidades de Évora e Elvas.

A Classe II, terá uma repartição mais heterogénea, com expressão sobretudo no Norte, abrangendo o Minho, determinadas bolsas de Trás-os-Montes, nas designadas terras quentes, assim como numa parte importante do Centro e do Litoral que se estende até perto da região de Lisboa. Entre a Figueira da Foz e algumas zonas mais abrigadas da influência dos ventos marítimos, nomeadamente nas regiões de Alcobaça e Caldas da Rainha, poderão encontrar-se áreas incluídas na Classe III e, em situações menos frequentes, na Classe IV.

A Classe III tem repartição dispersa no litoral, a sul de Aveiro, com expressão também no Ribatejo e Oeste, mas a sua mais extensa área encontra-se sobretudo a sul do Tejo, até aos contrafortes montanhosos do Algarve.

A Classe IV, inclui áreas dispersas nas zonas de influência da Classe III, em geral constituídas por condicionalismos protegidos da influência dos ventos dominantes ou das geadas mais intensas e duradoiras. Mas, é no Algarve que a Classe IV se encontra mais representada. Na realidade, nesta região, à excepção do barlavento algarvio, nas zonas mais expostas aos ventos marítimos e em algumas outras áreas também excessivamente expostas ou sujeitas a geadas, *C. capitata* encontra-se presente no estado adulto durante todo o ano, constituindo uma praga importante para as fruteiras que são mais susceptíveis aos seus ataques. Apesar disto, no Algarve, mesmo em áreas menos favoráveis para a mosca, dadas as distâncias em geral pequenas a percorrer, a invasão das populações de adultos permite que também nestas áreas possam ser elevados os prejuízos provocados nos períodos do ano em que as condições climáticas são menos adversas.

Nestas áreas, porém, os prejuízos não são maiores porque se sobrepõe ao clima um outro factor limitante da abundância de *C. capitata*: trata-se da carência de hospedeiros adequados ou da necessária sequência de períodos de maturação dos frutos.

Na Fig. 1 apresenta-se o climatograma das de: Montalegre e Castelo Branco (Classe I); Alcobaça (Classe II); e, Faro (Classe IV).

Relativamente à Classe I, as duas regiões consideradas (Montalegre e Castelo Branco) põem em evidência duas situações distintas. Para o caso de Montalegre a situação desfavorável para *C. capitata* resulta essencialmente da prolongada duração do período com temperaturas inferiores a 10ºC. No caso de Castelo Branco, essa situação resulta, por um lado, da temperatura baixa no Inverno e, por outro lado, da humidade relativa baixa (inferior a 60%) nos meses mais quentes do ano.

De salientar que *C. capitata*, apesar da sua reconhecida plasticidade ecológica, é uma espécie com afinidades subtropicais, evidenciando uma melhor capacidade de adaptação ás regiões cujas temperaturas são moderadas durante o Inverno.

Durante os períodos mais frios do ano, desde que a temperatura não baixe para valores inferiores aos limites que inibem a sua actividade metabólica (e que se situam próximo dos 10ºC), a longevidade dos adultos aumenta de forma muito acentuada. Para valores médios da temperatura de 35ºC a 18ºC, Bodenheimer (1951) indica valores para a longevidade dos adultos que vão desde 16 a 177 dias, respectivamente. A vida dos adultos depende, porém, em qualquer situação, da satisfação das necessidades alimentares.

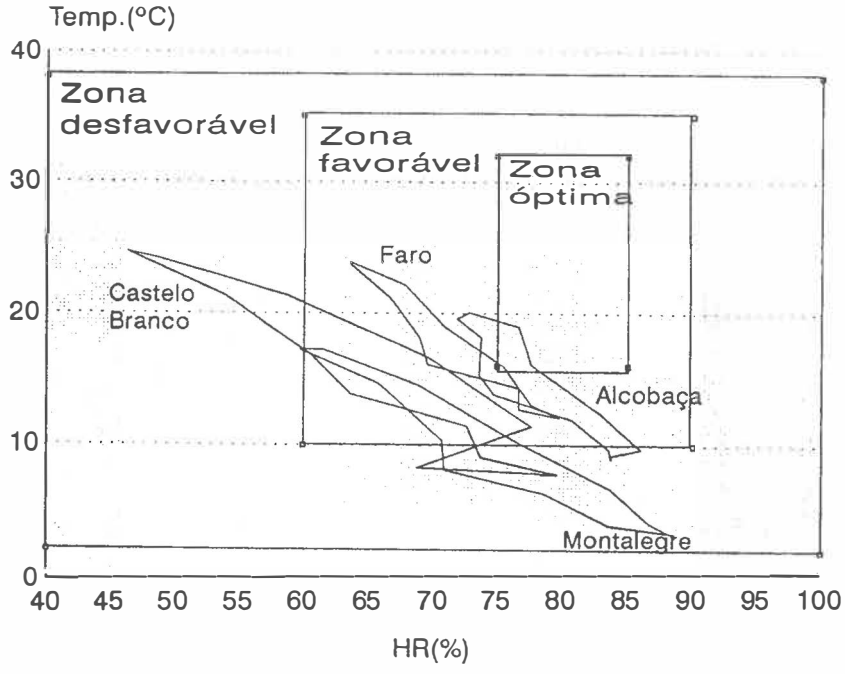

Fig. 1-Climatogramas correspondentes aos dados das estações meteorológicas de: Montalegre; Castelo Branco; Alcobaça; e Faro.

Quando as temperaturas são baixas e se aproximam de 0°C a mortalidade torna-se elevada e pode ser total, o que depende em grande parte da duração do período de tempo de exposição. Mas, em geral, quando se verificam temperaturas baixas, a mortalidade é atenuada pela alternância destas com os valores das temperaturas que ocorrem durante o dia.

A subordinação de *C. capitata* aos valores baixos da temperatura constitui a razão principal do deslocamento da ocorrência e da abundância relativa deste insecto para as regiões do sul. Isto não impede, porém, que possam surgir surtos ocasionais de infestações em regiões do Norte, como acontece em alguns anos no Minho, em pessegueiro, e também, em Trás-os-Montes, nesta cultura e em macieira. Esses surtos relacionam-se com a longevidade de *C. capitata* em tempo frio e com a existência local de condições microclimáticas particulares.

Dando-se razão a Cheikh (1969), a sobrevivência de *C. capitata* encontra-se assim dependente de três factores fundamentais: os valores das temperaturas baixas, a duração do período de exposição a estas temperaturas e as disponibilidades de alimento.

2.2-A influência do hospedeiro

A alimentação dos adultos constitui um requisito indispensável para que estes possam dispor da energia de que carecem por forma a manterem em tempo frio a sua actividade metabólica e, dentro dos limites térmicos favoráveis à sua existência, para que possam atingir a maturação sexual e tenham possibilidades de acasalarem, reproduzirem e de exercerem outras actividades normais, como a procura de hospedeiros, os movimentos gerais no habitat e os vôos de dispersão.

Em condições climáticas favoráveis para a biologia de *C. capitata*, a falta de alimentação dos adultos conduz a uma quebra drástica da sua longevidade (em geral superior a um mês) para 3 a 5 dias apenas, ou um pouco mais com a simples disponibilidade de água. Por esta razão, como é bem sabido, a alimentação dos adultos constitui uma necessidade essencial nas criações laboratoriais e mesmo crítica nas criações em massa, exigindo a preparação de dietas adequadas para se evitarem mortalidades antecipadas e permitirem a manutenção da capacidade reprodutiva de *C. capitata* (Vargas, 1989).

Nas condições naturais, os adultos encontram o alimento no néctar de flores, nas secreções das plantas e nas meladas excretadas por homópteros, dispondo as fêmeas ainda de um importante suplemento alimentar nos líquidos que se libertam dos frutos quando estes são picados pelo oviscapto durante o processo de postura (Bodenheimer, 1951; Vieira, 1952; Averill & Prokopy, 1989; Greany, 1989; Rice, 1989; Carvalho & Fernandes, 1993).

Estas noções elementares sobre a relação entre as condições climáticas, sobretudo no que se refere à temperatura, e à alimentação dos adultos, tem interesse ser recordadas para se compreender melhor a estreita dependência de *C. capitata* dos factores ecológicos e, se quisermos ir mais longe, para se interpretar a influência que estes factores exercem sobre o potencial biológico desta espécie. Mas, tendo como pano de fundo as considerações anteriores, é a relação entre *C. capitata* e os seus hospedeiros (no que se refere à sua abundância, sequência da frutificação, maturação dos frutos e susceptibilidade aos ataques), que assume a importância mais relevante numa perspectiva epidemiológica.

Sabe-se que a polifagia de *C. capitata* é muito elevada e que os citrinos constituem em Portugal continental o hospedeiro onde esta praga provoca os maiores prejuízos, apesar da aparente contradição de ser ,entre as fruteiras susceptíveis aos ataques, aquela em cujos frutos se verifica nos estados imaturos (ovos e larvas) uma mortalidade mais elevada, atingindo valores que no conjunto destes dois estados são vulgarmente superiores a 97% (Back & Pemberton, 1915; Greany, 1989).

Nestas circunstâncias, a importância que *C. capitata* tem como praga dos citrinos é atribuível principalmente à sua abundância populacional nas plantações e às consequências da actividade das fêmeas nos frutos.

Contribuem para essa abundância e suas consequências as características das plantações devido à estrutura e fisionomia das plantas e aos microclimas que estas geram (com efectiva importância nos períodos mais quentes e secos do ano). A conjugação destes factores com os tropismos que influenciam os movimentos dos adultos, contribuem também para a forte atracção a que estes se subordinam em relação aos citrinos, conduzindo-os a concentrarem-se e a permanecerem nestas plantações.

Ao nível das plantas, os adultos são atraídos pelas meladas e outras substâncias alimentares, assim como as fêmeas pelos frutos em fase de amadurecimento e, principalmente, por estes quando já se encontram maduros. Os frutos estimulam e permitem as posturas, com todas as consequências nocivas, resultantes das feridas abertas pelas picadas (repetidas num mesmo fruto ou afectando numerosos outros) e do subsequente desenvolvimento da descendência quando os ovos são depositados nas câmaras de postura.

Além destas razões, há ainda uma outra que ajuda a explicar a importância que *C. capitata* tem em relação aos citrinos. Não é por acaso que a principal área citrícola do País se situa no Algarve. O zero vegetativo para os citrinos encontra-se próximo dos 12,8ºC, valor que não se afasta muito do limite térmico inferior para o desenvolvimento de *C. capitata*. Nesta região a temperatura média anual é de cerca de 17ºC, com uma média no Verão um pouco superior a 22ºC. Por sua vez, o Inverno pode-se considerar suave, não atingindo em geral durante períodos prolongados valores inferiores a 0ºC, os quais são críticos para os citrinos e para *C. capitata*. Favorecem a cultura de citrinos e *C. capitata* o regime pluviométrico com valores baixos à volta de 600 mm, por ocorrerem principalmente no Inverno. O extenso período mais quente e seco durante aproximadamente 7 meses no ano constitui, porém, um factor desfavorável, mas a obrigatoriedade do recurso à rega nestas condições, para satisfazer as exigências fisiológicas das plantas, acaba também por favorecer *C. capitata*.

Na realidade, entre os citrinos e *C. capitata* existe uma certa afinidade quanto a exigências climáticas, embora as suas origens geográficas sejam consideradas diferentes. No entanto encontram-se no Algarve as melhores condições climáticas para manifestarem as suas potencialidades, vegetativas no caso dos citrinos e epidemiológicas no caso de *C. capitata*. E, quando se analisa a área de distribuição dos citrinos em Portugal continental vamos encontrar ,de norte a sul, estas plantas em maior abundância e concentração em regiões com condicionalismos climáticos que também podem permitir a ocorrência da mosca. Há assim um notável paralelismo na susceptibilidade dos citrinos e de *C. capitata* aos factores climáticos.

Nestas condições, a maior concentração de citrinos no Algarve explica em grande parte a importância que a praga tem em relação a esta cultura. Isto não significa, porém, que no Algarve os citrinos sejam os hospedeiros mais vulneráveis aos ataques, se compararmos com o que se passa com os pessegueiros, nectarinas, damasqueiros e figueiras.

De certo modo, as maiores facilidades de desenvolvimento e a mais elevada taxa de sobrevivência de *C. capitata* que se verifica nos frutos destas plantas, reflecte-se na maior abundância populacional nas plantações de citrinos durante o Verão e início do Outono. Esta abundância ocorre nos citrinos, com destaque para a "Valencia Late", ainda que a colheita dos frutos tenha sido realizada cedo.

Há, assim, como que um reforço nas populações que se desenvolvem nos citrinos à custa dos seus frutos ,e que se relaciona não só com a elevada taxa de sobrevivência de *C. capitata* na sequência dos frutos dos mencionados hospedeiros que vão amadurecendo ao longo do ano, como também pelas facilidades que os adultos têm de se deslocarem na procura de condições favoráveis, microclimáticas, de abrigo e de alimento. Nestas condições, ocorrem migrações para os citrinos, as quais são mais evidentes nos finais de

Setembro, quando as temperaturas ainda são elevadas e já se verificaram ataques vulgarmente intensos nos figos.

A elevada abundância populacional de *C. capitata* nos citrinos no final do Verão tem consequências preocupantes em relação à expansão que se está a verificar nas variedades de citrinos mais temporãs. E, se isto acontece em relação aos citrinos, para as outras fruteiras mencionadas, principalmente para os pessegueiros de maturação tardia e damasqueiros, a mosca do Mediterrâneo constitui um dos mais sérios obstáculos à sua expansão, a não ser que se continue a enveredar pela via da luta química, mas com todos os sérios inconvenientes a que esta conduz.

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ESTUDO PRELIMINAR DE *CERATITIS CAPITATA* WIED. EM FIGUEIRAS NA REGIÃO DE ALCOBAÇA

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INTRODUÇÃO

Quando em 1992 procedíamos à monotorização das populações da cigarrinha *Ficocyba ficaria* (HORVÁTH, 1897) em figueiras na Região de Alcobaça, deparámos com a presença de *Ceratitis capitata,* não apenas nas armadilhas mas também em figos de diferentes variedades.

Dada a importância económica desta espécie e tendo-se constatado que não existia qualquer estudo incidindo sobre *Ceratitis capitata* em figueiras, na região, iniciámos de imediato o estudo da sua evolução nesta fruteira ao longo da época activa.

Saliente-se que até 1988 *Ceratitis capitata* não estava instalada na Região de Alcobaça. Aparecia mas sem carácter permanente e os tratamentos eram esporádicos (GOUCHO, com. pessoal, 1988). Actualmente pode dizer-se que a mosca da fruta já se estabeleceu manifestando-se com intensidade diferente nas diversas fruteiras. Esta permanência será devida a alterações climáticas que ocorreram entretanto e/ou à maior abundância ou melhor qualidade dos frutos.

MATERIAL E MÉTODOS

As observações tiveram lugar num pomar de noventa figueiras situado a 1 Km de Alcobaça, onde seleccionámos nove exemplares das variedades "Pingo-de-Mel" e "Bêbera Preta", e em seis figueiras isoladas compreendendo as seguintes variedades: "Pingo-de-Mel", "São João Branca", "Do Norte", "Verdeal" e "Olho-de-Perdiz".

Foram utilizadas armadilhas cromotrópicas de cor amarela com cola, na densidade de uma armadilha por árvore. Cada armadilha era colocada no interior da árvore com o lado da cola voltado para o sul e substituída todas as semanas no mesmo dia (Foto Nº1).

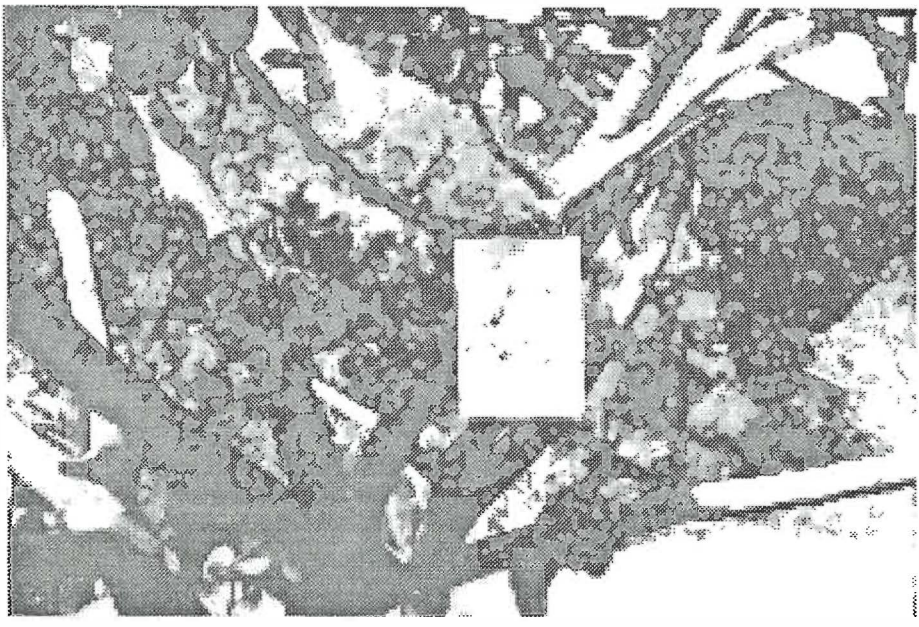

Foto nº1 - Armadilha no interior da árvore

Colhiam-se até dez futos por semana.

No laboratório, as armadilhas que tinham sido retiradas das árvores em estudo, eram colocadas individualmente em tabuleiros com petróleo durante duas horas, tempo suficiente para a dissolução da cola. Seguidamente passava-se o conteudo de cada tabuleiro para um funil de plástico com filtro de nylon de onde os insectos eram retirados para caixas de Petri.

Procedia-se então à contagem dos machos e das fêmeas de *Ceratitis capitata* por armadilha, utizando a lupa binocular WILD.

Os frutos eram colocados em caixas cobertas com tecido de nylon, anotando-se o tempo de duração de cada fase do ciclo de vida de *Ceratitis capitata*.

RESULTADOS

As condições favoráveis ao desenvolvimento de *Ceratitis capitata* vão de Junho a princípios de Dezembro. No entanto em figueiras e nos anos em estudo (épocas activas de 1992 e 1993) o seu aparecimento iniciou-se em Agosto, provavelmente devido ao amadurecimento dos frutos ocorrer no Verão em quase todas as variedades, sendo de notar que não verificámos quaisquer danos em figos lampos cuja maturação se dá em Junho.

Seria de muita utilidade fazer um estudo da dinâmica das populações de *Ceratitis capitata* mas não era esse o objectivo do trabalho pois o que pretendíamos era, nesta primeira parte, determinar a época em que *Ceratitis capitata* inicia a sua actividade em cada uma das variedades de *Ficus carica* L. (Tabela 1).

	ANOS	
VARIEDADES	1992	1993
Pingo-de-Mel	1 de Set	21 de Set
Pingo-de-Mel	15 de Set	31 de Ago
Pingo-de-Mel	15 de Set	31 de Ago
Pingo-de-Mel	15 de Set	21 de Set
Pingo-de-Mel	15 de Set	8 de Set
Pingo-de-Mel	15 de Set	8 de Set
Pingo-de-Mel	22 de Set	27 de Set
Pingo-de-Mel	22 de Set	14 de Set
Bêbera Preta	8 de Set	27 de Out
Bêbera Preta	29 de Set	28 de Ago
S. João Branca	11 de Ago	31 de Ago
Verdeal	8 de Set	8 de Set
Olho-de-Perdiz	4 de Ago	8 de Set
Do Norte	22 de Set	8 de Set

Tabela 1 - Inicio da actividade de *Ceratitis capitata* em cada variedade de *Ficus carica*.

Na tabela 2 encontram-se registados os valores percentuais relativos ás capturas efectuadas nos meses em que se verificou o início da actividade de *Ceratitis capitata*.

MESES	PERCENTAGENS(%)
AGOSTO	22
SETEMBRO	75
OUTUBRO	3

Tabela 2 - Valores percentuais de capturas por mês.

A regra para o inicio da actividade de *C. capitata* em figueiras na Região de Alcobaça parece ser quando o verão já vai avançado, fim de Agosto ou o mês de Setembro, dependendo ainda do microclima do local onde a variedade se encontra instalada.

Do total observado é de admitir que a relação macho / fêmea em armadilhas é de 1:1.

O número de figueiras que nos foi possível seleccionar para este estudo não permite determinar o aparecimento de *C. capitata* por variedade.

Nos frutos estudados em laboratório verificámos que a relação macho / fêmea é equivalente. Exemplificamos com alguns frutos da variedade "Pingo-de-Mel" (Tabela 3).

FRUTOS	Nº MACHOS	Nº FÊMEAS	TOTAL
1º	12	13	25
2º	3	4	7
3º	4	6	10

Tabela 3 - Número de machos e de fêmeas em três frutos da variedade "Pingo-de-Mel".

Relativamente ao primeiro fruto foi observada a fêmea a introduzir os ovos no fruto com este ainda na árvore (CELESTE COELHO, com. pessoal) admitindo-se que o desenvolvimento nesta variedade se tenha dado com a seguinte variação:

-> Período de incubação dos ovos -------- 10 dias

-> Fase larvar ---------- 16 dias

-> Fase pupal ---------- 10 dias

Relativamente ao número de frutos infestados não apresentamos comentários uma vez que em 1992, por razões que não pudemos ultrapassar, as observações efectuadas foram em número reduzido, e em 1993 ocorreram chuvas precoces que deterioraram os figos.

Quanto às preferências de *C. capitata* em relação às diferentes variedades de figueiras, nota-se maior incidência em "Bêbera Preta" provavelmente devido à menor espessura da epiderme, logo seguida de "Pingo-de-Mel" (Foto nº2).

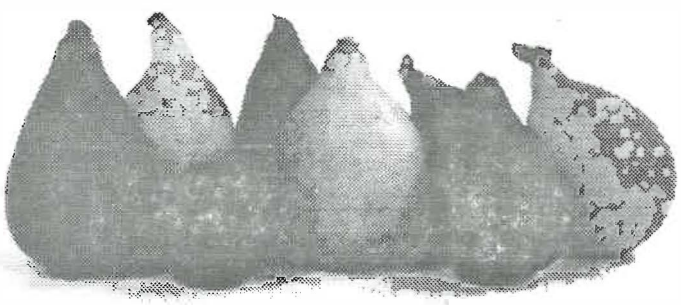

Foto nº2 - Frutos das variedades "Bêbera Preta"
e "Pingo-de-Mel"

ESPÉCIES ACOMPANHANTES

Tentando conhecer as biocenoses em que se integrava, elaborámos uma lista dos principais grupos que acompanhavam *Ceratitis capitata* nas variedades de *Ficus carica* em estudo, esperando encontrar alguns dos seus inimigos naturais.

De Agosto até Novembro capturaram-se os seguintes grupos:

-> Homópteros (Psilas, Cigarrinhas, Afídeos e Aleurodídeos)
-> Heterópteros (*Orius*, *Anthocoris*, Mirideos)
-> Tisanópteros (*Aeolothrips*)
-> Neurópteros (*Chrysopa*, *Hemerobius*, *Conwentzia*)
-> Coleópteros (Coccinelídeos, principalmente *Stethorus punctillum*, Estafilinídeos, Carabídeos, Cantarídeos)
-> Himenópteros (Braconidae, Proctotrupidae, Chalcidoidae)
-> Dípteros (Lonchaeidae e Sirfídeos, outros)
-> Aranhas
-> Ácaros (*Panonychus ulmi*, *Tetranychus telarius*)

Em Dezembro apenas foram capturados alguns Dípteros, Cigarrinhas e Coleópteros.

CONCLUSÕES

Os resultados observados justificam a continuação e o aprofundamento dos estudos do comportamento de *Ceratitis capitata* nas variedades de *Ficus carica* que apresentam maior significado económico com vista a processos de protecção integrada desta fruteira.

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EVOLUTION OF FRUIT FLY (*CERATITIS CAPITATA* WIED) POPULATION IN FOLLOWING HOSTS PEACH TREES, APPLE TREES AND CITRUS TREES IN SANTARÉM (PORTUGAL)

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The fruit fly, originally from Western Africa, has now spread throughout all continents as a result of the transportation by man of its eggs and larvae in fruits. The high polyphagia and adaptative capacity to new ecological conditions make this pest a great calamity to world fruit growing. In favourable years attacks start in medlars, damask plums, peaches, figs oranges and apples as well. Although peaches are its favourite hosts where it is considered as a key-pest as well as citrus fruits, significant attacks in apples as been registered. This study of the evolution of adult flight, based on the weekly cathing of adults in McPhail glass traps with a 4% water solution of ammonium phosphate, took place between 1988 and 1990, following hosts cultures (peach, apple and citrus trees) in the agricultural exploitation of High School of Agriculture of Santarém as a subject of practical courses in order to assess the risk of attack and support the decision about the oportunity of the pest management methods against this pest. By observation of the percentage of affected fruits, a correlation between the level of catches per trap and per day can be established as well as the damages caused. A brief analysis of the influence of the temperature, relative moisture and rainfall in the flight of *Ceratitis capitata* can be made too. The level of auxiliary insects catched in the trap throughout the observation period was mainly increased of *Chrysopidae* and *Syrphidae*, important predators of aphids and other phytophagans although McPhail traps are more selective than yellow traps and less detrimental to beneficial species.

Effets de la cyromazine sur la fécondité et fertilité chez *Ceratitis capitata* Wied. (Diptera, Tephritidae)

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Parmi les régulateurs de croissance d'insectes (RCI), la cyromazine (N-cyclopropyl-1,3,5-triazine-2,4,6-triamine) a montré une activité excellente sur les diptères comme *Musca domestica* Linnaeus, *Culex pipiens* Linnaeus, *Aedes aegypti* (Linnaeus) ou *Liriomyza trifolii* (Burguess), entre autres. Ces resultats nous ont amené à tester l'effet de la cyromazine sur *Ceratitis capitata* Wied. en conditions de laboratoire.

La cyromazine c'est une triamine qui agit en tant que régulateur de croissance d'insecte. C'est un R.C.I. de synthèse connu sous le nom commercial de Trigar. Cette molécule provoque des anomalies morpho-anatomiques chez la larve et la pupe des diptères et affecte pourtant son développement. Entre les aspects plus intéressants du mode d'action de ces regulateurs de croissance il faut considerer les effets sur la reproduction.

L'objectif général de l'étude présentée est établir si l'adittion de la cyromazine par voie alimentaire a des effets sur la fécondité de l'adulte de *C. capitata* et sur la fertilité des oeufs pondus, et si ces effets sont concentration dépendants.

Nous avons étudié les effets de troisconcentrations croissantes (0,1 mg/ml, 1 mg/ml, 10 mg/ml) de ce produit sur la fécondité et fertilité des adultes de *C. capitata* nourris avec une diète dans laquelle on ajoute la cyromazine.

Les effets du traitement sont observés sur la longevité des adultes, sur le nombre d'oeufs pondus et le rythme de ponte, sur le développement embryonnaire ainsi comme sur l'éclosion des larves.

Effets sur la longevité des adultes alimentées avec cyromazine.

L'application de la cyromazine par voie alimentaire sur l'adulte n'a pas des effets létaux sur ce diptère. Dans tous les cas tudiés nous avons trouvé que 15 jours aprés l'émergence des adultes, un 40-50% des adultes sont encore vivants. 50 jours aprés l'émergence entre un 35-45% des adultes vive encore. La cyromazine ajouté a l'alimentation n'a pas des effets sur la longevité des adultes pour aucuné des concentrations et temps d'alimentation testées.

Effets sur le nombre d'oeufs pondus

Par contre, l'application voie alimentaire provoque une réduction dans le nombre d'oeufs pondus, c'est à dire, du potentiel reproducteur. Le nombre moyen d'oeufs pondus par une femelle pendant les 15 premiers jours dans le témoin varie entre 170 et 236 selon la repetition tandis que dans les lots traités varie entre 110 et 150.

Pour les concentrations testées nous n'avons pu trouver un effet concentration dépendant. Les valeurs obtenus pour chaque concentration sont trés similaires entre eux et différs de la memme façon du témoin. La durée de la période d'alimentation avec la cyromazine ne semble pas etablir des différences et soit quel soit cette période, les effets sont trés similaires.

Effets sur le rythme de ponte des oeufs

Nous n'avons pas trouvé des différences dans le rythme de ponte; il est trés constante dans le témoin et les traités pour tous les cas de figures et répétitions étudies. Dans tous les cas on attend un 50% de la ponte vers le 4ème-5ème jour de ponte, et un 80-85% vers le 8ème jour de ponte.

Effets sur l'éclosion des larves

Le pourcentage d'eclosion de les larves différs de façon assez important entre le témoin et les lots traités. Cet pourcentage est, en moyen, de 89% dans le témoin tandis que pour les traités varie entre le 49% et le 90% selon la concentration testée. L'alimentation des adultes avec la concentration de 10 mg/ml provoque une réduction trés important dans le nombre de larves et pourtant des populations de *C. capitata*. Nous avons trouvé aussi des différences dans la durée du développement embryonnaire. Pour les témoins et les traités avec 0,1 mg/ml et 1 mg/ml, nous avons le 80% d'eclosion 48-72 heures aprés la ponte, tandis que pour la concentration de 10 mg/ml, le développement embryonaire est plus long, entre 72-120 heures.

D'aprés ces resultats nous pouvons conclure que la cyromazine est un régulateur de croissance assez active sur la mouche mediterranéen des fruits. Nos experiénces montrent que l'addition de cyromazine dans la alimentattion de l'adulte de *C. capitata* a des effets sur la fécondité et fertilité de la femelle. Nous avons trouvé une réduction important dans le nombre d'oeufs pondus ainsi comme dans le pourcentage d'eclosion et pourtant dans le nombre de larves obtenues. La cyromazine provoque pourtant une réduction important des populations de *C. capitata*. Il est necessaire de poursuivre cette étude pour mesurer la valeur exacte de cette réduction en fonction de la concentration.

Two North American Rhagoletis spp. in Europe

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During a taxonomic survey on tephritid species occurring in Switzerland a Ph.D. student of the ETH Zürich examined various private and public insect collections. In the collections of the museum for Natural History at Luzern and of the cantonal museum of natural history in Lugano he found specimens of *Rhagoletis completa* Cresson and of *Rhagoletis indifferens* Curran that had been caught between 1983 and 1990 in light traps operated in the canton Ticino (Merz, 1991). Six months later a similar report was published in 1991 in Italy indicating a wider distribution of *R.completa* in the Veneto and Friuli Venezia-Giulia region (Duso 1991)

A subsequent survey of the Swiss Federal Quarantine Service with yellow traps conducted in 1991-1993 confirmed the occurrence of higher population densities of *R. completa* throughout the southern part of the canton Ticino (Sottoceneri and Sopraceneri). The existence of only 2 small populations of *R. indifferens* at the original collection sites near Lugano and at Meride (Sottoceneri) in low population densities and localised distribution was also confirmed. Trapping operations at the international airports of Zürich and Geneva produced negative results. Based on this preliminary survey it can be concluded that the occurrence of both species is probably limited to the Canton Ticino.

The question is raised whether systematic surveys should be conducted to investigate not only the precise distribution of the two species but also focus on the potential introduction of other important fruit fly species such as *Rhagoletis pomonella.*

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USE OF PARASITOIDS (HYM.) IN THE CONTROL OF FRUIT FLIES (DIP.: TEPHRITIDAE) IN ARGENTINA: BIBLIOGRAPHIC REVIEW (1937 - 1991)

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Summary

The authors provide a thorough review on the existing literature on fruit fly parasitoids used in integrated pest management programs in Argentina between 1937 and 1991.

They comment on the surveys made in the country, different methods to rear and propagated the species, and biological control projects which incude introduction, release and recovery of exotic species.

They provide a description of what is being done in the country at this moment, and update lists of native and exotic species.

1 - Introduction

The fruit flies constitute a serious world pest, which affects nearly all the fruit crops. The direct damage (larvae in fruits) or indirect damage (export limitations) can result in a considerable loss of money; and this pest can even cause the disappearance of a whole fruit area (Aluja, 1984).

In Argentina the fruit flies are represented by a species complex belonging to Tephritidae family. Their great capacity to adapt to any environment allows them to develop in almost all the country. Thus, it is possible to find them in tropical, subtropical, desertic, and cold areas.

Among the complex of fruit flies we can mention two important species. One is *Ceratitis capitata* (Wied.) (Mediterranean fruit fly or medfly, as it is commonly known in English) of African origin and introduced in South America in about 1905 (Gallo et al., 1970). The other is *Anastrepha fraterculus* (Wied.) (South American fruit fly) which belong to a New World genus; it is the most widely distributed species of the genus occurring from Texas, USA to Chile and Argentina (Enkerlin et al., 1989).

From an economic point of view, these two species are the most important ones because they cause the worst damage to Argentinian fruit crops (Turica & Mallo, 1958; Turica et al., 1971; Nasca et al., 1981).

Despite the numerous insecticide treatments against fruit flies, the annual loss through direct damage varies between 15 % and 20 % of the Argentinian fruit produce. This figure is increased by the loss caused by the fruit low quality. Besides, we have to consider the risks and consequences related to the use of chemicals (poisoning, pollution, and the onset of new pests, among the most important ones) (INTA, 1992). The world growing tendency to reject the chemicals in food as well as in the environment will result, hopefully, in their complete substitution or at least significant reduction in their use.

In Argentina this pest restricts the exports of fruit and vegetables to various countries.

Over 250 host plants that allow the pest development have been recorded in Argentina (Alvarado & Ritacco, 1991). The pest preferably attacks stone fruit (peaches,

plums, apricot, etc.) but it can also cause serious damage to citric fruits (orange, tangerine, grapefruits) and also pears, apples, etc. Vegetables such as tomatoes and pepper are also frequently attacked.

According to Turica (1968) the main occurrence of fruit fly natural parasitoidism is located in areas with tropical or subtropical climates where a wide variety of wild fruits abound. Such is the case of certain regions in Asia, Africa, America and Australia. In South America, one of the parasitoid center lies in a vast area wich includes the south of Brazil, north of Argentina and some minor regions in north-west Uruguay. Apart from these two areas, we can mention the one in the north of Brazil (Amazon) (Silva et al., 1992) and the one in Mexico (Turica, 1968).

Within Argentina there are two major *Anastrepha* parasitoid areas, one of them corresponding to the "yungas" forests in the northern provinces of Tucuman, Salta and Jujuy; and the forests of Misiones, Corrientes and Entre Rios (Turica & Mallo, 1961).

The disappeareance of a vast part of the forests due to the cutting down of trees to provide new areas for crops, and the abuse of chemicals has resulted in the reduction of the main parasitoid centers in America (Turica & Mallo, 1958; Turica, 1958).

2 - Bibliographic Review

The biological control projects of fruit flies in Argentina have always been undertaken within Integrated Pest Management (IPM) programs mainly by Instituto Nacional de Tecnología Agropecuaria (INTA) of Castelar in Buenos Aires and by Estación Experimental Agrícola "Obispo Colombres" (EEAOC), Facultad de Agronomía y Zootecnia (FAZ) and by Centro de Investigaciones Para la Regulación de las Poblaciones de Organismos Nocivos (CIRPON) at Tucumán.

One of the most exhaustive studies on fruit flies parasitoid survey, percentage and geographic distribution of species in Argentina has been produced by Turica & Mallo (1961), who worked in several Argentinian citric areas for three years (1958-1960). The result showed a low percentage of parasitoidism. These areas covered the North West and North East of the country as well as the littoral and central part of Buenos Aires.

Within the NW, the province of Tucumán was one of the most widely surveyed provinces, although most papers on fruit fly parasitoids refer only to their occurrence, introduction and host records (Blanchard, 1938a, 1938b, 1938c; Schultz, 1938; Hayward, 1940a, 1940b; Domato & Aramayo, 1947; Ratkovich, 1950b; Turica & Mallo, 1958, 1961; Nasca & Terán, 1969; Nasca, 1973; Fernandez de Araoz & Nasca, 1984). Turica & Mallo (1961) and Turica (1968) make brief biological comments about some species. Brethes (1924); Blanchard (1947, 1966); De Santis (1965, 1967); Fischer (1963, 1965); Loiácono (1981); Wharton & Marsh (1978); Wharton & Gilstrap (1983) and Wharton (1988) provided taxonomic studies on the parasitoids recorded in Argentina.

The first step towards the obtention, rearing and release of fruit fly native parasitoids in Argentina were taken in the thirties. Ogloblin (1937) describes a very easy method to obtain and protect the parasitoids and reports about the first studies made with fruit fly parasitoids at the Laboratorio de Entomología de la Estación Experimental de Loreto (Misiones). Schultz (1938) states that the EEAOC released three species of native parasitoids (*Doryctobracon tucumanus* (Turica y Mallo), *Ganaspis pelleranoi* (Brethes) and *Eucoila* sp.) through 1938 in Tucumán citric orchards.

One of the earliest pionners that used the native parasitoids against fruit flies was Mr. K. J. Hayward who recommended the obtention, rearing and release of parasitoids. Between 1940 and 1944 he released two native parasitoid species (*D. tucumanus* and *G.*

pelleranoi) in field crops in Tucumán (Hayward, 1940b, 1941, 1942, 1943, 1944a, 1944b). Hayward's laboratory method was based on the obtention of parasitoids from fruit fly cocoons collected in wild fruits and their release in citric orchards later on, and the use of a fly-trap ("pozo trampa") in the soil for the fruits. This trap permits only the emergence of the parasitoids. This fly-trap was very similar to that used by Ogloblin in 1937 (see Hayward, 1940a). These methods were also used by Turica (1968) in Corrientes and Misiones. Nasca et al. (1980) also used and modified this fly-trap.

In 1942, three species of parasitoids (*D. tucumanus*, *G. pelleranoi* and *Utetes (Bracanastrepha) anastrephae* (Viereck)) were sent from the Tucumán region to Perú to control *A. fraterculus*. Only one of the released parasitoids (*G. pelleranoi*) was established on *A. stricta* (Clausen, 1978; Wharton, 1989b).

The first project in the biological control of fruit flies in Argentina was the introduction of *Tetrastichus giffardianus* Silvestri during 1947, and was undertaken by the Ministerio de Agricultura de la Nación. The species introduced were sent from Brazil to the Estación Experimental in Concordia (Entre Ríos) where the parasitoid was reared and realesed in citric orchards (Turica, 1968). In 1947 *T. giffardianus* was released in Tucumán but did not establish (Ratkovich, 1950a).

In the sixties, the Departamento de Patología Vegetal del INTA at Castelar succesfully propagated *C. capitata*. Consequently, various parasitoids species were introduced from Mexico in 1961, namely *Biosteres arisanus* (Sonan) (= *Opius oophilus*), egg-larval parasitoid; *Doryctobracon crawfordi* (Viereck); *Diachasmimorpha longicaudata* (Ashmead) (= *Biosteres longicaudatus*) and *Aceratoneuromyia indica* (Silvestri), larva-pupal parasitoid (Crouzel & Terán, per. comm.). A culture of the last two mentioned parasitoids was established at the INTA Castelar and their releases were made in different fruitcrop areas in Tucumán, Jujuy, Misiones and Córdoba. Further recoveries showed 18 % to 35 % of parasitoidism (Turica, 1968). At the begining of 1968 another species was introduced in Argentina, in this case a pupal parasitoid, *Pachycrepoideus vindemmiae* (Rondani) which was sent by OIRSA (Organismo Internacional Regional de Sanidad Vegetal) Costa Rica. This species was latter sent by INTA Castelar to Tucumán (Nasca et al., 1981).

In 1966 and 1967, *A. indica* and *B. arisanus* were sent by INTA Castelar to Tucumán. The former species was realesed in four sites of the province for four years and it became well established (Nasca & Terán, 1969; Nasca, 1973). The latter species was realesed only once but was not recovered in further surveys (Nasca & Terán, 1969).

A. indica was again introduced in Tucumán in the seventies, and this time the parasitoid shipment came directly from Mexico (Nasca, 1977).

During the years 1973 and 1977 *D. longicaudata* and again *P. vindemmiae* were introduced through INTA Castelar in Tucumán. Both parasitoids together with *A. indica* were reared at the insectary of FAZ and later released in different areas of Tucumán and Catamarca (Nasca, 1977). There is no further evaluation of these releases.

In 1986, *D. longicaudata* and *A. indica* were again introduced in Tucumán trough the CIRPON from Costa Rica. A culture of these two species was propagated and also one of *P. vindemmiae* (CIRPON, 1987/1988). Releases of *D. longicaudata* and *P. vindemmiae* in forest areas of Tucumán were carried out by the CIRPON between 1986 and 1988. There is no further evaluation.

Native parasitoid lists of fruit flies in Argentina were published by De Santis (1941, 1967); Ratkovich (1950b); Turica & Mallo (1958, 1961); De Santis & Esquivel (1966); Turica (1968), and Rosillo & Portillo (1971). According to these author lists 26 parasitoid autochtonous species were recorded in Argentina; they belong to the families

Braconidae (15), Eucoilidae (3), Pteromalidae (4), Chalcididae (2) and Diapriidae (2). Some of these species were never described, others represent new synonyms or new combinations (De Santis, 1965, 1967; Loiácono, 1981; Wharton & Marsh, 1978; Wharton & Gilstrap, 1983; Wharton, 1988, 1989a). In Table 1 are listed the native parasitoid valid species in Argentina and the synonyms and combinations used before.

Table 1: List of native valid species of tephritid parasitoids recorded in Argentina. Synonyms and previously used combinations.

Family	Species	Status
Braconidae:		
	Doryctobracon areolatus (Szépligeti)	
	Biosteres, Parachasma	G
	Opius cereus Gahan	S
	Opius saopaulensis Fischer	S
	Doryctobracon brasiliensis (Szépligeti)	
	Biosteres, Opius, Parachasma, Diachasmoides	G
	Coeloides anastrephae Brethes	S
	Opius (Diachasma) brasilianus Fischer	S
	Doryctobracon tucumanus (Turica y Mallo)	
	Opius, Diachasmoides	G
	Opius bellus Gahan	
	Opius turicai Blanchard	S
	Opius gomesi Costa Lima	S
	Opius trimaculatus Spinola	
	Opiellus	G
	Utetes (Bracanastrepha) anastrephae (Viereck)	
	Opius, Bracanastrepha	G
	Opius mombinpraeoptantis Fischer	S
Eucoilidae:		
	Ganaspis pelleranoi (Brethes)	
	Eucoila	G
	Rhoptromeris haywardi (Blanchard)	
	Eucoila	G
Diapriidae:		
	Coptera haywardi Loicano (Ogloblin i.l.)	
	Galesus, Psilus	G
	Trichopria (Planopria) anastrephae Costa Lima	

S = synonym ; G = previously used generic combination.

3 - Discussion

Six exotic parasitoid species have been introduced in Argentina to control fruit flies, especially *C. capitata*, most of which came from Mexico. Only three of them are known

to have established (see Table 2). Unfortunately, the studies made on the other species lacked consistency and evaluation, as Clausen (1978) states.

Among the parasitoids introduced and established is *P. vindemmiae* (see Table 2). This poliphagous and cosmopolitan species, which occasionally attacks tephritid pupae (Wharton, 1989a), was already present in Argentina under other names (De Santis, 1941; Hayward, 1943) -which are synonyms at present (De Santis, 1979; De Santis & Fidalgo, in press)- when it was introduced through an official project.

Numerous authors have published papers on fruit fly native parasitoids in Argentina. However, none of them have produced a comprehensive study regarding taxonomic or biological basic aspects, like life cycle or life span, mating behaviour, immature stages, etc.; rearing methods and impact on hosts. This information is essential to carry out fruit fly control programs successfully.

Little is known about the actual numbers of parasitoids that exist in natural populations and the numbers of parasitoids that will have to be released (by aumentative or inundative technique) to achieve rates of parasitoidism that would supress fruit fly populations to tolerable levels (Knipling, 1992).

Several authors have indicated that biological control alone is not the single solution for the control of fruit flies (Turica & Mallo, 1958; Turica et al., 1971; Aluja, 1984; Wharton, 1989a; Mitchell, 1990). But biological control can be complementary to other methods, for example, to the Sterile Insect Technique (SIT) (Wharton, 1989a), this technique alone is not practical when the population density of the target pest is high (Knipling, 1992).

At present, the goverment of San Juan and Mendoza are carring out an erradication program against *C. capitata* using SIT. This project is supported by INTA and the Comisión Nacional de Energía Atómica (CNEA) (L. Alvarado, per. comm.).

At the CIRPON the senior author is carring out studies on the fruit flies parasitoids, particulary field surveys and biological research on native species toward their use in IPM and Erradication programs.

Table 2: Introduced and released parasitoids used to control tephritid pests in Argentina.

Family & Species	Established	Introduced from	References
Braconidae			
Biosteres arisanus (Sonan)	Non	México	Nasca & Terán (1969), Crouzel & Terán (per.comm.)
Diachasmimorpha longicaudata (Ashmead)	Yes	México	Turica (1968)
Doryctobracon crawfordi (Viereck)*	--	México	Crouzel & Terán (per.comm.)
Eulophidae			
Aceratoneuromyia indica (Silvestri)	Yes	México	Turica (1968), Nasca (1973)
Pteromalidae			
Pachycrepoideus vindemmiae (Rondani)	Yes	Costa Rica	Turica (1968)
Tetrastichidae			
Tetrastichus giffardianus Silvestri	Non	Brasil	Ratkovich (1950a) Turica (1968)

* This species have not been recovered yet (Crouzel & Terán, per. comm.).

4 - Conclusion

It is necessary to carry out new surveys and research on native parasitoids in order to:

A) Confirm which of the exotic species introduced have established well and, according to results, repeat the introduction of those which failed to establish or introduce new species in order to increase parasitoidism levels. Thus, we ned more intensive pre-release studies and more critical selection of natural enemies adapted to local conditions.

B) Evaluate native parasitoid action, so as to use the species in control projects, thus supporting IPM programs or Erradication programs through SIT. This information will be useful not only for Argentina but also for other areas where fruit fly problems occur.

Currently it is believed that parasitoid as well as sterile fly release integration may well be the most effective and practical method from an ecological point of view and the most acceptable solution to the problems caused by fruit flies in various parts of the world (Knipling, 1992).

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Host Marking Pheromones in Fruit Flies: State of the art

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1. Historic background

Investigations on host marking pheromones (HMP) of fruit flies were initiated and are still most advanced in the temperate *Rhagoletis* spp. Ideas and methods generated by this research have stimulated similar investigations in *Ceratitis capitata* and *Anastrepha* spp. On the other hand it seems that *Bactrocera (Dacus)* do not produce and deposit HMPs as oviposition-deterrents (ODP) (Prokopy et al. 1989).

There are three distinct periods in HMP research on *Rhagoletis:* Early observations (first report by Prokopy, 1972) on basic biological characteristics of ODP in *R. pomonella* (such as influence on behaviour, physiological investigations on production site etc.) were conducted in the 1970's mainly by Prokopy et al. and stimulated similar research on *R. completa* in California (Cirio, 1972) and *R. cerasi* in Switzerland (Katso-yannos, 1975). Comprehensive reviews have been published by Prokopy 1981; Boller 1981; Averill and Prokopy, 1989.

The second period - starting in 1975 and lasting until 1987 - covered the first field applications of ODP against *R. cerasi* with crude extracts that achieved a degree of protection of the treated cherries of over 90% (Katsoyannos & Boller, 1976, 1980). After 15 years of research the chemical structure of the HMP was identified in 1985 and the compound synthesised in 1987 by Swiss investigators. (Hurter et al.,1987; Ernst & Wagner, 1989).

The third phase started a few years ago. It focuses on the influence of HMPs on the foraging behaviour of flies under laboratory, semi-field and field conditions and addresses the basic question how HMPs are best allocated in orchards to achieve optimum protection of the fruits. Larger field experiments with the synthetic natural pheromone of *R. cerasi* started in 1988 and continued in 1989 with a synthetic modification of the pheromone that has been submitted in 1993 as first ODP world-wide for official registration in Switzerland (Boller & Aluja, 1992; Aluja & Boller, 1992a, b).

2. Examples of recent and current research programs

2.1 Foraging behaviour of *R. cerasi* as influenced by HMP

Field-cage experiments carried out between 1985 and 1990 in Switzerland involving single cherry trees and entire cherry orchards showed interesting behavioural patterns: Flies landing on cherries with complete HMP treatment of foliage and fruit exhibited a significantly reduced residence time on the tree compared to the time of flies landing on untreated trees or trees where only the cherries had been treated. However, those flies landing on trees with full HMP treatment could no longer discriminate HMP treated fruit due to constant exposure of their tarsal sensillae to the HMP on the leaf surface. Hence it was concluded that optimal HMP application should aim at treating only the fruits and if necessary only the underside of the leaves. Foliage should be left untreated whenever feasible in order to allow the HMP sensilla to recover quickly after HMP contact (Aluja & Boller, 1992a,b).

Experiments were conducted to explore the feasibility to apply HMP only to one half of the tree crown whereby the trees were divided either vertically or horizontally. In the former case only the South and East quadrants of the trees were sprayed with HMP, whereas in the latter case only the lower part of the tree crown was treated. The results were surprising. In the case of vertical division the treated quadrants could be protected against oviposition with an effectiveness of 95% but the infestation rate of cherries in the untreated parts was only 1/3 of the expected value. This strongly suggests that flies landing on HMP treated trees do not move horizontally within the tree in search for untreated resources. Where only the lower part was treated the infestation rate of cherries in the untreated upper part was as high as in untreated check trees whereas the treated lower part could be protected against oviposition. This is suggestive that flies landing on HMP treated trees start an upward movement in search of HMP free resources (Aluja & Boller, 1992a).

The most recent experiments carried out between 1990 and 1992 investigated the effect of optimal HMP allocation on cherry fruit fly infestation patterns and control efficiency in commercial cherry

orchards in Switzerland. In one case (1990) 24 cherry trees (4 rows of 6 trees) were included in the experiment and sprayed with 5 litres of a 10ppm HMP solution per tree. The second tree in each row remained untreated (= 4 check trees). It was speculated that the infestation rate would build up with increasing distance to the untreated tress due to frequent encounters with HMP treated resources and increasing ovarian pressure. The opposite was true. Highest infestation levels were of course observed on the untreated check trees (17%) and between 2.3% and 5.7% of infestation was observed in the trees in their immediate neighbourhood. The infestation rates at the distant corners of the orchard were very low (<1%).These results suggest that flies with constant contact with HMP treated resources ca refrain from oviposition and move around within a patch of host trees in search for suitable oviposition sites much longer than anticipated. In our experiments they did locate the few untreated trees, concentrated on their fruits until fly density and/or natural HMP intensity exceeded a critical threshold. Then they moved to adjacent HMP treated trees accepting the treated cherries reluctantly as second choice. These experiments indicate that HMP application should be combined with efficient trapping on and around untreated catch-trees.

Further field experiments utilised this approach and cherries could be protected with an efficiency of >90% even under adverse meteorological conditions. Based on these field data the synthetic pheromone was submitted to the Swiss registration authorities for official registration.

2.2 Response of *Ceratitis capitata* of different geographic regions to a standard HMP solution.

The response of the medfly to its HMP has been investigated on and off by various scientists in Europe and the USA after the existence of the HMP had been reported for this species by Prokopy, Ziegler & Wong (1978). In Europe preliminary laboratory and field experiments were conducted from 1983-86 in Switzerland, Italy (Sardinia) and Greece in the framework of an IOBC co-ordinated research project. The results obtained both in field and laboratory experiments were highly inconsistent, unpredictable and not reproducible at other locations indicating various interfering factors such as inadequate laboratory bioassays, influence of host fruit characteristics and possible influence of geographic origin of the given medfly strains. Best results were achieved in the Swiss laboratory with medfly material from Kenya whereas flies from Sardinia seemed to exhibit a different behavioural pattern (Boller, unpublished IOBC report).

When the Global IOBC fruit fly working group was established in Rome (1987) it was decided to start a new co-ordinated research project addressing the question of variability in HMP perception by medfly strains of different geographic origins. Seven collaborating fruit fly laboratories (Argentina, Brazil, Greece, Reunion Islands/France, Mexico, Switzerland and the USA) utilised an improved standard laboratory bioassay (Boller & Aluja, 1992) and a standardised HMP stock solution provided by the Swiss laboratory. The results of this joint project are now available and have been submitted for publication (Boller et al. 1994). It can be concluded that all geographic populations react the same way to a standard HMP solution prepared from medflies of Mediterranean origin. Comparative results of 3 medfly strains with different rearing history (Kenya F50, Sardinia F38 and Seibersdorf F500) show that the Kenya strain had fully retained its HMP discrimination abilities (discrimination coefficient DC = 76.8) and host marking behaviour. The performance of the two other strains was reduced (Sardinia DC 47.7; Seibersdorf DC 43.5%) and their dragging lasted only a few seconds. However, it is remarkable that even the medfly strain with the longest rearing history of the world (Seibersdorf) has still retained a residual capability to discriminate HMP marked fruits. These results indicate a genetic plasticity and robustness of the medfly confirming earlier observations in the field of quality control (Boller & Calkins, 1984).

Recent investigations of Papaji et al. (1989a,b, 1990, 1992) show that the medfly can not only be deterred from oviposition by HMP marked oviposition sites but can also be stimulated by HMP to lay eggs into existing oviposition punctures. However, the authors conclude that HMP still remains an interesting potential tool in the management of medfly populations if the proper concentrations are applied. Taking note of these advances in HMP biology the Global fruit fly group has decided to continue laboratory and field investigations to explore further this potential. The current project is co-ordinated by F. Diaz-Fleischer of the Moscamed Program at Tapachula, Mexico and is assisted in Europe by the Swiss laboratory at Wädenswil (E. Boller). The main objective is to conduct simple experiments both in field-cages and the field with natural populations with HMP extracts provided to the participants.

3. Conclusions and outlook

Research on the chemistry and deployment strategies of HMP in ***Rhagoletis*** spp. is likely to continue and to produce new insight into the practical potentials of HMP application in IPM programs. HMPs of *Rhagoletis* might therefore become the pacemakers of a future HMP technology.

It is anticipated that the co-ordinated IOBC activities in progress on ***Ceratitis capitata*** in the laboratory and under field conditions will produce important base-line data in the near future that might help to clarify certain contradicting phenomena in the relationship of the medfly and its host marking pheromone. One of the interesting question that remains to be clarified is the knowledge of the conditions that are inducing to change the oviposition-deterring HMP into a signal acting as oviposition stimulant. A prerequisite for a chemical identification of the ODP is the development of a reliable electrophysiological technique for *Ceratitis capitata* that has encountered so far many problems (Cmjar; Städler, personal communication). But in consideration of the cosmopolitan nature and economic importance of the medfly such investigations might be justified.

Investigations on HMP of ***Anastrepha*** spp. are in progress in various Latin American countries. Here we can expect interesting new insight into the HMP - fruit fly interactions that might stimulate the interest of chemists to take a closer look at the chemistry in this important fruit fly complex. This could potentially lead to novel strategies of biotechnical fruit fly control.

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SOME OBSERVATIONS ON ACANTHIOPHILUS HELIANTI (ROSSI) (DIPTERA, TEPHRITIDAE) IN A CARTHAMUS FIELD OF BASILICATA (SOUTH ITALY).

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Summary

In a Chartamus field (Carthamus tinctorius L.) of Basilicata (South Italy) an infestation by Acanthiophilus helianti was observed. About twenty different cultivars were present. Dissected samples showed the presence of the insect, as well as flies emerged from stored samples. By means of yellow traps in the field the presence of adults was also sometime observed.

1 - Introduction

The economic importance of the Tephritidae group is mainly related to Fruit Flies, widely distributed in the world with many pest species. Many other species of this family (about 46%) appear associated to flowers. Some of them can be considered useful as reservoir of entomophagous species, as Myopites stylata on Inula viscosa, host of Eupelmus urozonus chalcidoid of Olive fruit Fly (Fimiani & Digilio, in Press). Some others appear to be a pest as a species of Terellia present in Basilicata on Cynara cardunculus.

Among them Acanthiophilus helianti (Rossi) is recorded as sofflawer pest (Carthamus tinectorius). It belongs to subfamily Tephritinae, with species mainly characterized by anthophagy.

As pest of safflower is reported for Egypt, India, Iraq, Israel, Romania, Russia and Turkey (White & Elson-Harris, 1992), Central Europe, and Canary Islands (Belcari, 1985). In Italy was first described by Bezzi (1792) as Musca helianthi, found on Heliantus tuberosus.

In this Country was later indicated for North, Center and South Italy, as well as Sardinia and Sicily (Belcari, 1985). Infestations are recently reported for some experimental areas of Pulia, Tuscany and Friuli (Ippolito& Parenzan, 1983, 1986, 1990 a,b ; Ricci & Ciriciofolo, 1983; Zandigiacomo & Iob, 1991).

Geografic distribution of this pest on Carthamus is reported in the Fig.1.

2 - Materials and Method

The observation were carried out in an experimental safflower field where the Dipartimento di Produzione Vegetale dell'Università di Basilicata used different cultivars of Carthamus tinctorius to study seeding density and other agronomic parameters. In some occasions they furnished samples, offering the opportunity to observe possible infesting insects.

Very few aphides and rarely coleoptera present were not considered. As the main infestation was due to Acanthiophilus helianti, the flower heads were placed in some emergence boxes, to obtain adults.

In other cases 10 of them were isolated each in a single little glass-box, to see the adult distribution among them.

Considere cultivar: 1) Agsof 01- 2) Agsof 02- 3) Agsof 4)Belisario- 5)Benno- 6)Boemondo 7) Bonello- 8) Britto- 9) Elena- 10) Guaimaro-11) NA- 12) Rainulfo- 13) Roberto- 14) Ruggiero- 15) Soffloca 541-16) Soffloca 918- 17), 18),19) unknown cultivar "x" "y" "z".

Table I - N° of males and females from signle Flower heads

	Infested	M	F	T	Healthy
Agsøt	3a	7	9	16	
	3b	2	2	4	
tot	**2**	**9**	**11**	**20**	**8**
Boemondo	6a	2	0	2	
	6b	5	2	7	
	6c	3	0	3	
	6d	4	1	5	
tot	**4**	**14**	**3**	**17**	**6**
Britto	8a	14	4	18	
tot	**1**	**14**	**4**	**18**	**9**
Guaimaro	10a	1	2	3	
	10b	2	0	2	
	10c	3	2	5	
tot	**3**	**6**	**4**	**10**	**7**
NA	11a	3	2	5	
tot	**1**	**3**	**2**	**5**	**9**
Ruggero	14a	3	3	6	
tot	**1**	**3**	**3**	**6**	**9**
Cv " X "	17a	1	0	1	
	17b	3	4	7	
tot	**2**	**4**	**4**	**8**	**8**
Cv " Y "	18a	2	2	4	
	18b	1	1	2	
	18c	3	2	5	
tot	**3**	**6**	**5**	**11**	**7**

3 -Results

While certain samples of flower heads did not give adult flies, in some periods a good emergence was obtained.

Fig. 2 is refered to 13 samples of 10 heads each, i. e 130 in total, and shows the distribution of 242 adults (114 males and 128 females) among 13 cultivars (Average of 1.86 flies per head).

Fig. 3 is refered to 13 samples of 30 heads each, i. e. 380 in total and shows the distribution of 810 adults (385 males and 425 females) among 13 cultivars (Average of 2.03 flies per head). Some cultivars are represented in both the samples.
An idea of the infestation of single flower head is given in the table I where among many normal of them, the infested ones are indicated with emerged flies.
To obtain data on presence of adults in the field, 11 yellow square traps were employed. Considered the fixed position in report to the growing plants very few catches were obtaines. The cumulative number of catched flieds is showed in Fig. 4.

4 - Conclusions

An infestation of the Tephritid Acanthiophilus helianti was reported on Carthamus tinctorius, in a safflower area of Basilicata. Considering the future of this oil seed plant, the flower pests reach economic importance. It appears useful, besides the fuit flies of economic importance to consider also some flower flies.

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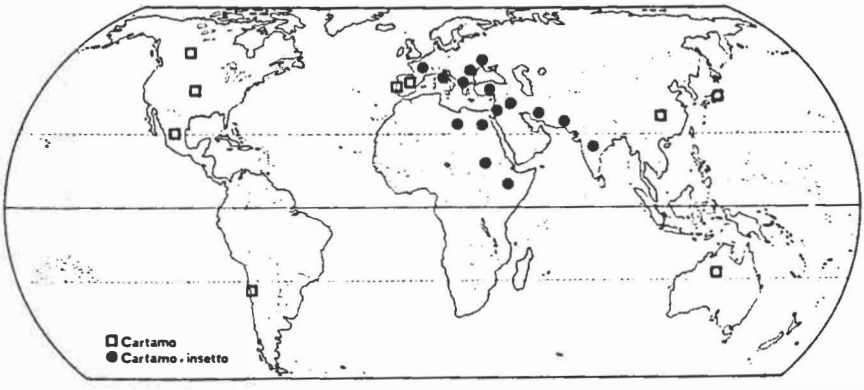

Fig. 1. Geografic ditribution of *A. helianti* Rossi on *Chartamus* (Ricci Ciriciofolo, 1983).

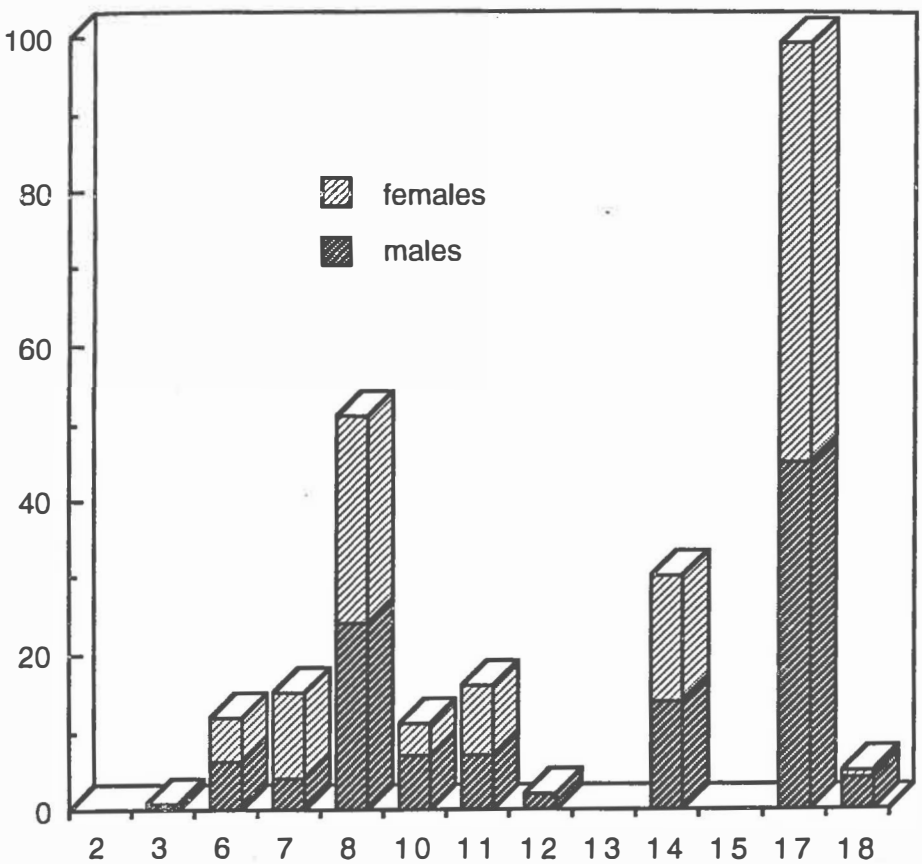

Fig. 2. Emergence of adults (male and female) from 13 samples of 10 head flowers, (June 1988)

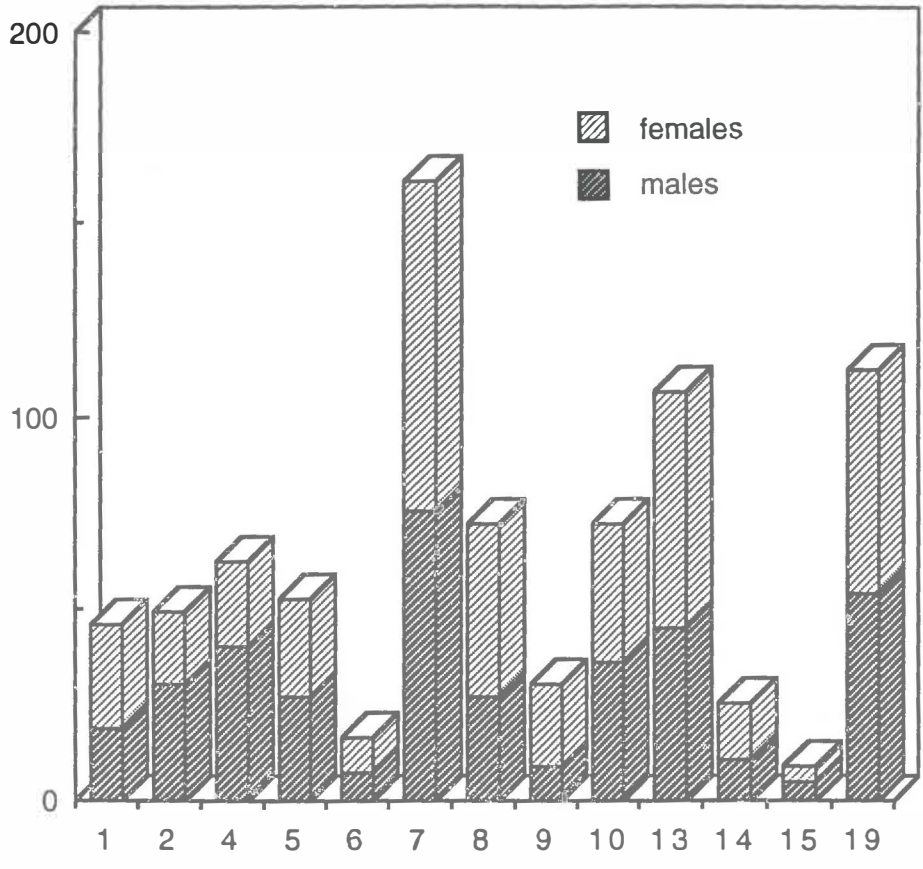

Fig. 3. Emergence of adults (male and females) from 13 samples of 30 head flowers) (July, 1989)

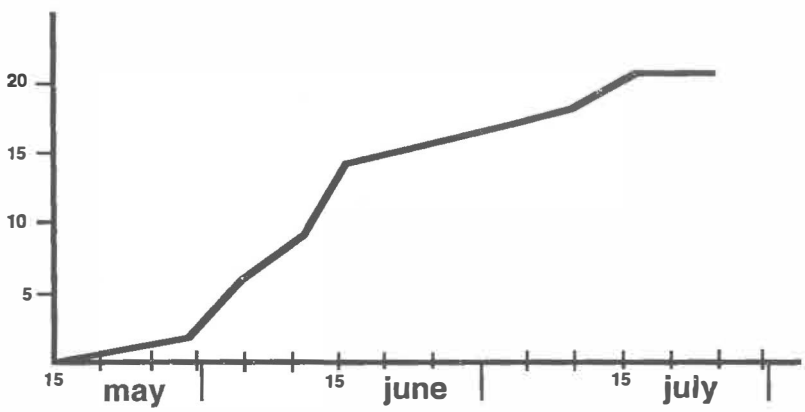

Fig. 4. Cumulative number of adults catched by 11 yellow traps (1990)

OBSERVATIONS ON REARING *EUPELMUS UROZONUS* DALM., PARASITOID OF *BACTROCERA OLEAE* (GMEL.), ON *CERATITIS CAPITATA* (WIED.)

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Summary

Eupelmus urozonus, ectophagous parasitoid of the olive fly, was bred in the laboratory on *Ceratitis capitata*. The parasitoid females perforate the *Ceratitis* puparium with their ovipositors through the spiracular plates, depositing an egg on the pupa or, more rarely, bound to the inner wall of the puparium by a complete cocoon-like covering of fine, silky hairs lashed transversely across it. Apart from sugary substances, their feed on the haemolymph of the host by forming a feeding tube. Only 30% of the females succeed in laying their eggs on the factitious host (*C. capitata*). At 25°C, the pre-oviposition and longevity periods proved to be 13 and 38 days , respectively, and fecundity 63 eggs per female (from 14 to 212). Male development cycle lasted 49.3 ± 3.9 days, and female 51.1 ± 3.9. The percentage of adults emerged from the parasitized pupae proved to average 40%. The small number of egg-laying females and the low development percentage of pre-imaginal stages indicate poor adaptation of *E. urozonus* to the substitute host. Nevertheless, it would seem possible to mass-produce the parasitoid at low cost for use in biological control against the olive fly, after having verified efficiency and lack of harmful effects due to a tendency to hyperparasitism.

1 - Introduction

Eupelmus urozonus Dalm. (Hym., Eupelmidae) is an example of species which may develop as primary ectoparasitoid or as ectoparasitoid of a great range of other primary parasitoids. Hosts attacked include Lepidoptera, Diptera (especially Cecidomyidae and Tephritidae) (DELANOUE & ARAMBOURG, 1965; ASKEW, 1961), Coleoptera and gall-forming Hymenoptera (mostly Cynipidae). The Eupelmid has been obtained as well from various species of Hymenoptera parasitoids and from the Neuroptera *Chrysoperla carnea* (Steph.).

E. urozonus is particularly known as parasitoid of *Bactrocera oleae* (Gmel.), the olive fly, but, on the olive, it has also been obtained from *Phloeotribus scarabeoides* Bern. (olive bark beetle) and *Prays oleae*

(Bern.) (olive kernel borer). Furthermore, it has often been found in olive agroecosystem as parasite of *Myopites stylata* F., Tephritid which causes galls on the inflorescence of *Inula viscosa*.

E. urozonus is found on the olive fly from August to the beginning of autumn, when some researchers affirm that it leaves its host to parasitize others (particularly *M. stylata*) (ARAMBOURG & PRALAVORIO, 1974; RUSSO, 1967). Recently, we have observed in Sardinia that *E. urozonus* parasitizes *B. oleae* up to November and winters in diapause in the larval stage on the drupes both on the tree and on the ground. It prefers to attack the mature larvae of the olive fly, but can also live on the I and II instars and behave like a psudoendophagous on the pupae (ARAMBOURG & PRALAVORIO, 1974). *Eupelmus* parasitism varies considerably from grove to grove as well as from year to year. Normally, the parasitism is surpassed by that of another Chalcidoid *Pnigalio mediterraneus* Ferr. *et* Del., but predominates in certain Italian, French and Greek areas (RUSSO, 1967; ARAMBOURG & PRALAVORIO, 1974; LOUSKAS *et al.*, 1980), probably because of the tendency to hyperparasitize this Chalcidid.

Although the ectophagous parasitoids of the olive fly in some cases cause a high percentage of mortality, they are normally unable to restrain the populations in autunm (DELRIO & CAVALLORO, 1977; DELRIO & PROTA, 1976; LOUSKAS *et al.*, 1980). This has been mainly attributed to the small initial parasitoid populations, depending upon the alternative hosts available in spring. Rearing *Eupelmus urozonus* on the substitute host *Ceratitis capitata* (Wied.) in the laboratory (ARAMBOURG, 1964) opens the road to the possibility of biological control of the olive fly with inundative release methods. In this paper, observations are reported on the biology of *Eupelmus* made during the mass-rearing on *Ceratitis* pupae in the laboratory.

2 - Materials and Methods

Eupelmus rearing on *Ceratitis* pupae (the latter reared on artificial medium) was effected by taking ca. 200 emerged adults from olives attacked by *B. oleae*. The *Eupelmus* were kept in a plexiglass cage (40 cm x 40 cm x 40 cm) and fed with solid sugar, water being given separately. The top of the cage contained 6 slots (25 cm x 1.5 cm), through which were inserted perpendicularly rectangular plexiglass slides (24.5 cm x 12 cm) with the *Ceratitis* pupae to be parasitized. Liquid, non-toxic paper glue was spread over the slide surfaces, and ca. 400 *Ceratitis* pupae were showered onto each slid before the glue dried. Each cage contained ca. 1500 *Eupelmus* adults and ca. 2400 *Ceratitis* pupae, which were removed after 1 day with a small paint-brush and then preserved in plastic containers. To avoid inducing the diapause, the

parasitized *Ceratitis* pupae were kept in thermostatic chambers at 25°C with photoperiod D:L 10:14h (LOUSKAS & LAUDÉHO, 1977).

To study oviposition, longevity and fecundity, cylindrical cages (5 cm diam. x 8.5 cm hight) were used, containing 2 males and 1 virgin female, to which were made available 30 pupae a day for parasitization. The pupae were examined externally and opened to reveal the silken threads, tubercles, punctures, feeding tubes and eggs. The observations were made at three different constant temperatures (20 °C, 25 °C and 28 °C) and for 14h photoperiod.

3 - Results

3.1 Behaviour of the female

Eupelmus adults generally mated during the day of emergence, drinking the water and feeding upon the solid sugar placed at their disposal. The females ran continuously all over the internal surfaces of the cage until they found the *Ceratitis* puparia, tapping and drumming upon them with the tips of their antennae. When they located the spiracular plates they inserted their ovipositors, pumping them up and down and occasionally moving the gaster to either side or raising and lowering it. The majority of the females preferred to penetrate through the posterior spiracles; only 7% penetrations took place through the anterior spiracles. The females had great difficulty in perforating the *Ceratitis* puparium; out of more than a hundred observed females, only ca. 30% succeeded in ovipositing in the undamaged puparia. Undoubtedly, the damaged puparia greatly attracted the females which were then able to lay their eggs freely in them; withered pupae, however, did not allow the larvae to conclude their development.

The puncture determined a blackish scar on the pupa near the abdominal extremity, or, more rarelly, on the antennae, legs and pterotechas. The females fed upon the haemolymph of the host, utilyzing the ovipositor to construct a feeding-tube thereby putting the pupa in communication with the external surface of the puparium. This feeding upon the pupa could be repeated several times in successive days.

The egg was laid in two different ways: (1) in 87% of cases, deposited on the body of the pupa without any attachment (on the abdomen, in the space between the pterotecha and base of the abdomen, or between the tips of the legs and antennae); and (2) in 13% of cases, bound to the inner wall of the puparium near the spiracles by a complete cocoon-like covering of fine, silken threats lashed transversely across it. Normally, one egg per pupa was laid, but some females could lay several eggs in the same puparium up to a maximum of 5. The foregoing could have been due to the difficulty in perforating

the puparia; a female with a high superparasitizing tendency laid its eggs singularly in puparia artificially preperforated with a microneedle near the spiracles.

After laying the egg, the female withdrew its ovipositor leaving a small tubercle of solidified material on the external surface of the puparium (on the perforated spiracle). Also, in 31% of cases, a small plug formed of fine, silk-like treads, of the same material supporting the egg, was left on the external surface of the puparium. The tubercle and/or the plug enabled parasitized pupae to be easily identified.

3.2 Effect of host age

The pupae of *Ceratitis capitata* do not constitute a natural host for *Eupelmus urozonus*, since they are found in nature a few centimetres below the ground surface, where they are inaccessible to the parasitoid. Pupal susceptibility to parasitism was tested by placing pupae of different ages in the cages on successive days. Parasitism occurred at all pupal ages, varying from 69.5% to 95.4%, but *Eupelmus* adult emergence resulted inferior (21.9% to 43.6%), indicating poor response of the host to parasitoid development (Tab. I). Particularly, in *Ceratitis* pupae close to emergence (last 2 days of life) the percentage of adult parasitoid development was very small (8.4 - 9.4%). While 50% of the *Eupelmus* larvae in the young pupae died at an advanced stage, in the older pupae ca. 80% of the *Eupelmus* larvae did not develop beyond the first larval stages.

3.3 Influence of temperature on longevity, fecundity and development

The lifespan of *Eupelmus* adults decreased with the increase in temperature (20 to 28 °C), showing large individual variations. Females lived longer than the males at all the experimental temperatures.

Table I - Effect of age of *Ceratitis capitata* pupae on parasitic performance of *Eupelmus urozonus* (27 °C).

Pupal age (days)	No. of pupae tested	% of parasitized pupae	% of *Eupelmus* emergence
1	100	76.0	23.0
2	100	84.0	35.0
3	100	79.0	42.0
4	100	69.5	21.9
5	100	87.7	28.3
6	100	93.3	34.2
7	100	95.4	43.6

The lifespan of the females averaged 38.4 d at 25°C against 17.1 for the males (Tab. II).

Table II - Effect of 3 constant temperatures on the longevity and fecundity of adults of *Eupelmus urozonus*.

Temperature (°C)	Longevity (days)		Fecundity (No. eggs/♀)
	Males	Females	
20	42.2 (10-57)	65.5 (15-80)	107.5 (42-206)
25	17.1 (4-29)	38.4 (9-58)	63.5 (14-212)
28	15.1 (9-27)	32.0 (10-47)	42.1 (6-125)

The preoviposition period shortened with the increase in temperature; at 20 °C, the average period was 13 days, whereas at 28 °C, it decreased to 2.3 days. The oviposition period followed a similar course ; at 20 °C, it averaged 30 days (with ca. 3,6 eggs per female per day), whereas at 28 °C, peak laying was reached in 10 days, whereupon laying slowed and, in some cases, stopped (Fig. 1). Total fecundity proved to be very variable, but a female maintained at 25 °C layed as many as 212 eggs. Normally the femals layed 1 egg per pupa in the presence of sufficient hosts (ca. 20 pupae per day per female). However, some females exhibited a tendency to superparasitism, laying up to 5 eggs per pupa. The developmental period of the egg into adult shortened with increase of temperature (50 days at 20 °C; ca. 20 days at 28 °C) (Tab. III). In general, emergence took a fairly long time (e.g., at 25 °C, the first individuals emerged after 21 days, whereas the last ones emerged 15 days later), the males developing more rapidly than the females.

Table III - Mean (± S.D.) developmental time (days) for *Eupelmus urozonus* at 3 constant temperatures.

Temperature (°C)	Males	Females
20	49.3 ± 3.9	51.2 ± 3.9
25	25.4 ± 2.7	28.0 ± 3.2
28	20.4 ± 3.4	23.1 ± 2.5

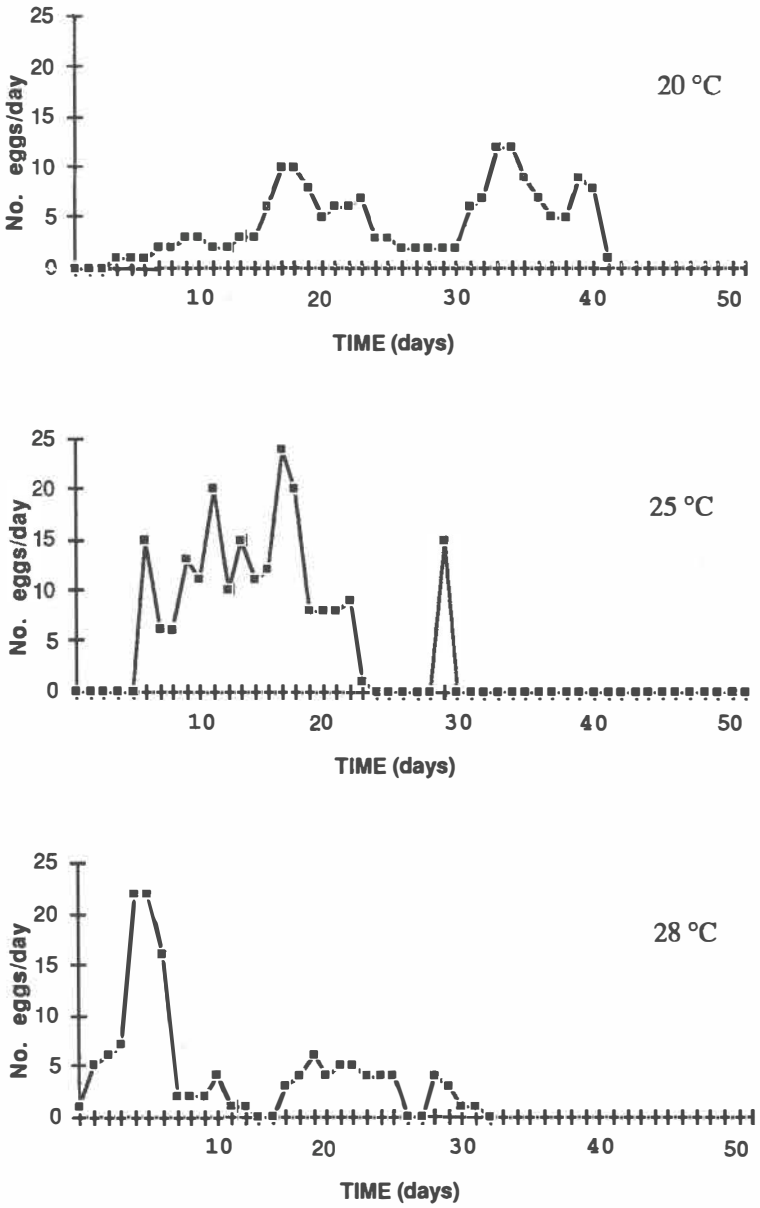

Fig. 1 - Oviposition trend of three females of *Eupelmus urozonus* at three costant temperatures.

4. Conclusions

The polyphagous behaviour of *E. urozonus* made it possible to utilize a substitute host in the laboratory. Mass rearing enabled *Eupelmus* biology to be studied in detail, forming the basis of biological control application against the olive fly. The reasons for choosing *Ceratitis capitata* pupae as host were its sistematic proximity to the olive fly and especially its economical mass rearing possibilities. However, our observations proved that *Ceratitis* pupae did not constitute an ideal host, because only a small number of females succeeded in penetrating the puparium and relatively few adults developped. Nevertheless, we managed to produce a laboratory colony which could be improved by the selection of a more suitable *Eupelmus* strain. Suppression of the diapause enabled ca. 12 generations to be obtained in a year.

The disponibility of *Eupelmus* will allow inundative releases in summer to control the olive fly. The advantage of using *Eupelmus* instead of *Opius concolor* , a parasitoid already used in biological control, is its parasitism of the young olive fly larvae, which have not yet seriously damaged the drupes. The pratical utilization of *Eupelmus*, however, requires careful field observations, not only regarding olive fly parasitism, but also its hyperparasitoidal behaviour on the beneficial insects (DELANOUE, 1964).

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EVALUATION OF FOOD ATTRACTANTS FOR MEDFLIES (Ceratitis capitata WIED, DIPTERA: TEPHRITIDAE) IN PLASTIC McPHAIL TRAPS.

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Summary

Three experiments were conducted in which three fly-attractants [Dacus bait^{R} (Db), Dacus bait^{R} -100 (Db-100) and Dacona^{R} (Da)] produced in Creece, were compared with two imported attractants; Nulure^{R} (Nl) and Buminal^{R} (B). The traps used were the Plastic McPhail ones with the lower half of yellow colour (International Pheromones) baited with water solution of 3% or 9% of the above attractants and Borax at 3%.The graduation of the five attractants in the three experiments were as follows:

1st. NL 9%, NL 3%, Db 3%, Da 3%.

2nd. NL 9%, NL 3%, Db 3%, Db-100 3%, Da 3%.

3th. The NL 9%, B 9% and Db 9% did not differ statistically, while the Db-100 9% and Da 9% were inferior.

It is obvious that the attractants Dacus bait at the dose of 9% showed the same attractivity as Nulure protein at the same dose.

1- Introduction

The Mediterranean fruit fly *Ceratits capitata* (Wiedemann) is a major agricultural pest in many areas in the world, attacking over 250 varieties of fruits, vegetables and nuts. The fly is present in Creece causing considerable damage to citrus fruits as well as to figs, apricots, apples, pears, peaches, pomegrants and other fruits and vegetables, mostly in the southern regions and islands of the country (Zervas, 1986).

The fly is controlled by cover or bait sprays. The bait for spraying is consisted of an insecticide (mostly Organophosphate) and an attractant (mostly protein hydrolysate). For survey and detection of the fly is mostly used the McPhail trap baited with an attractant. On the contrary the widely known abroad Delta trap baited with TML is seldom used, as the trap is expensive.

In this paper, the results, obtained in Attika in 1989 by comparing the efficiency of the attractants in Plastic McPhail traps, are presented. The purpose of this work was to compare the already known and the new attractants imported or produced in the country, in order to find the most convenient for detection and monitoring the Medfly, as well as to improve the bait sprays, by suggesting the most convenient attractants and for using them for the construction of lure and kill long lasting traps for the fly. (Zervas,1982)

2- Material and Methods

All experiments were conducted in the orchard of the Agricultural University of Athens in Votanicos. In this orchard citrus, fig, pomegranate and other fruit trees are cultivated and the Medfly could reach high population levels. The comparison of the attractants was conducted in Plastic McPhail traps , with lower half of yellow colour (International Pheromones). The capasity of the trap was 300 ml of water solution of the tested attractant . Five attractants were tested in the experiment as follows:

1. Buminal (B) (protein hydrolysate, produced by Bayer A.G. Germany).
2. Nulure (Nl) (protein hydrolysate, produced in USA.

3. Dacus bait (Db) (protein hydrolysate, produced by ALESIS, Thessaloniki, Creece).
4. Dacus bait-100 (Db-100) (protein hydrolysate, plus urea, produced by ALESIS).
5. Dacona (Da) (a product made from urea, by FYTOFIL. Attiki, Creece.)

The traps were checked weekly and the water solution of the attractants was changed. In each block, the position of the traps was rotated by one position to the other at the time in each collection date.

The first experiment was conducted from 18th of August to 18th of September 1989. All traps were suspended in fig trees and the attractants Dacus bait (Db) in 3% water dilution, Dacona (Da) in a 3% water solution and Nulure (Nl) in a 3% and 9% dilutions were used. 3% Borax was added in all the water solutions in order to preserve the caught in the traps insects as well as to promote further decomposition of the proteins (Zervas, 1984). The 3% water solution has been chosen in the experiments as this solution is suggested by the producers, exept Nulure in which the suggested by the producer dose is 9% of the dence product.

The second experiment was conducted from 18th of September to 23th of October 1989. The traps were suspended in citrus trees and the same atrractants were used as in the first experiment in the same dilutions as before. In this experiment, a new atrractant Dacus bait-100 in a dilution 3% plus 3% Borax was added, to be compared with the other attractants.

The third experiment was conducted from 23th of October to 12th of December 1989, in the same area. In this experiment, the attractivity of the atrractants Dacus bait, Dacus bait-100, Dacona and Nulure to the Medfly was compared. All the attractants were used at the dose of 9% plus 3% Borax as the attractivity of them to a dose of 3% has been proved to be inferior (unsatisfactory) of the 9% dose of Nl.

3- Results

In table I, the mean number of medflies caught in Plastic McPhail traps, baited with various attractants, is shown. It is obvious that Nulure at the dose of 9% solution showed the best attractivity than the other attractants tested, during the hole period of the five weeks. Also Nulure at a dose of 3% was superior for three weeks than Dacus bait and Dacona at the same dose. In the other two weeks the attractivity of Nulure, Dacus bait and Dacona was equal at the dose of 3%. Dacus bait and Dacona at the 3% dose showed almost equal attractivity to the fly.

According to the total catches of the attractants tested, expressed as a percentage of the total catches of Nulure at the dose of 9%, the attractants could be rated as follows: a) Nl 9%, b) Nl 3%, c) Db 3% and d) Da 3%.

In table II, the results of the second experiment are shown. In this experiment, a new attractant the Dacus bait-100 is included to the others which were the same as in the first experiment. The results were similar as in the previous experiment. The attraction of Nulure at the dose of 9% was significantly higher than the other attractants. Nulure at the dose of 3% showed the same attractivity as Dacus bait at 3%, but it was superior to Dacus bait-100 and Dacona at the same dose. The Dacus bait-100 showed an attractivity close to that of Dacona, but inferior to Dacus bait. This is probably due to the addition of urea in Dacus bait which redused the percentage of protein hydrolysate of the attractant. The rating of the tested attractants could be as follows: a) Nl 9%, b) Nl 3% and Db 3% equal, c) Db-100 3% and Da 3%.

In table III, the results of the third experiment are shown. The experiment lasted for 7 weeks. All the atrractants used at the dose of 9%. The attractant Buminal replaced Nulure 3%. The attractivity of Nulure, Dacus bait and Buminal had no statistical difference for seven weeks. The attractivity of Nulure didn't differ statisticaly in four of the seven weeks, compared to Dacus bait-100 and in three of the seven weeks, compared to Dacona. Easily the tested attractants could be rated as follows: a) Nulure 9%, Buminal 9% and Dacus bait showed the same attraction power, b) Dacus bait-100 9% and in the last place Dacona 9%.

Table I-Mean number of Medflies (*Ceratitis capitata*) cuptured in Plastic McPhail traps baited with various food attractants, in Attica Greece during 1989.

	x no of flies cuptured /Week/Trap Attractant			
Period 1989	Db 3%	Da 3%	NI 3%	NI 9%
18/8-22/8	176.5^{a}	47.0^{b}	209.5^{a}	$279.8^{a\ 1}$
22/8-29/8	$219,5^{b}$	169.8^{b}	440.5^{a}	303.5^{ab}
29/8-6/9	275.3^{bc}	256.5^{c}	375.0^{ab}	466.0^{a}
6/9-12/9	164.0^{b}	170.5^{b}	256.0^{b}	497.5^{a}
12/9-18/9	173.8^{c}	210.0^{bc}	260.8^{b}	397.0^{a}
TOTAL x 18/8-18/9	1009.1	853.8	1541.8	1943.8
% of NI 9%	52	44	80	100

Db=Dacus bait(R), Da=Dacona(R), NI=Nulure(R)
1. Within a row, numbers followed by the same letter are not significantly different (P>0.05) (Duncan's multiple range test).

Table II-Mean number of Med flies, (*Ceratitis capitata*), cuptured in Plastic McPhail traps baited with various food attractants in Attica Greece during 1989.

	x no flies captured / Week/Trap Attractant				
Period 1989	Db 3%	Db-100 3%	Da 3%	NI 3%	NI 9%
18/9-26/9	160.0^{b}	138.0^{b}	110.8^{b}	282.0^{a}	$378.8^{a\ 1}$
26/9-3/10	180.5^{b}	88.6^{c}	128.8^{bc}	138.3^{bc}	335.3^{a}
3/10-10/10	271.0^{b}	183.5^{bc}	149.0^{c}	204.0^{bc}	503.5^{a}
10/10-17/10	90.5^{ab}	95.0^{ab}	20.3^{b}	66.0^{b}	167.8^{a}
17/10-23/10	682.3^{a}	403.8^{b}	363.5^{b}	748.0^{a}	479.5^{ab}
TOTAL x 18/9-23/10	1383.8^{b}	908.8^{c}	772.3^{c}	1438.3^{b}	1865.0^{a}
% of NI 9%	74	49	41	77	100

Db=Dacus bait(R), Db-100=Dacus bait-100, Da=Dacona(R), NI=Nulure(R)
1. Within a row, numbers followed by the same letter are not significantly different (P>0.05) (Duncan's multiple range test).

Table III-Mean number of Med flies, (*Ceratitis capitata*), cuptured in Plastic McPhail traps baited with various food attractants in Attica Greece during 1989.

	x no flies captured / Week/Trap Attractant				
Period 1989	Db	Db-100	B	Da	NI
23/10-30/10	492.6^{ab}	665.8^{a}	561.0^{ab}	382.8^{b}	486.6ab[1]
30/10-6/11	237.2^{abc}	158.6^{c}	272.2^{ab}	173.2^{bc}	285.2^{a}
6/11-14/11	47.6^{c}	74.6^{bc}	124.0^{a}	106.4^{ab}	91.2^{ab}
14/11-20/11	63.8^{a}	47.8^{ab}	59.2^{ab}	29.2^{b}	54.2^{ab}
20/11-28/11	158.4^{a}	69.2^{c}	83.2^{bc}	112.0^{b}	155.6^{a}
28/11-4/12	1.8^{a}	3.6^{a}	1.2^{a}	2.0^{a}	1.4^{a}
4/12-12/12	2.2^{a}	2.2^{a}	1.4^{a}	1.0^{a}	1.2^{a}
TOTAL x 23/10-12/12	1003.6	1021.8	1102.2	806.6	1075.4
% of NI 9%	93	95	102	75	100

Db = Dacus bait$^{(R)}$, Db-100 = Dacus bait-100, B = Buminal, Da = Dacona$^{(R)}$, NI = Nulure$^{(R)}$

[1]. Within a row, numbers followed by the same letter are not significantly different (P>0.05) (Duncan's multiple range test).

4- Conclusions

Five products used as attractants for monitoring or controlling fruit flies as *Dacus oleae, Rhagoletis cerasi* and others, were tested in order to find their attraction to Medflies. The attractants Dacus bait, Dacus bait-100 and Dacona are produced in Creece and are used for controling Olive flies by bait sprays as well as for monitoring. The attractant Buminal is a product of Europe, produced in Germany, Spain and Italy (Delrio, 1984), while Nulure is a product of United States. Both of the last two attractants are not produced in Creece, so it is difficult to find them in the market and for experiments or for other uses (control).

The four attractants compared at the dose of 3% water solution were inferior to Nulure at the dose of 9% dilution. According to the results, the rating of the attractants was: NI 9%, NI 3%, Db 3%, Db-100 3% and Da 3%. On the contrary all the attractants at a dose of 9% showed a better attraction for the Medflies, so Nulure, Buminal and Dacus bait had no statistical difference, while Db-100 and Dacona were statistically different than the other three attractants. According to the rating of the attractants tested, it is obvious that the substances Nulure, Dacus bait and Buminal, which are consisted of protein hydrolysates, showed in all tested doses a superior attractivity to the substances Dacus bait-100 and Dacona, which have as base urea. The urea which produces only ammonia by microbial or chemical decomposition seems to be less attractive to Medflies. In other experiments, Zervas (1982) found that a 3% percentage of ammonium sulfate was less attractive to Olive and Med flies than Dacus bait and Buminal at the same dose.

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VITAMIN MIX REQUIREMENT OF THE OLIVE FRUIT FLY LARVAE

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Summary

An experiment was carried out to test the effect of three commercialy available vitamin mixtures upon the performance of the olive fruit fly, *Dacus oleae* (Diptera: Tephritidae), larvae reared in yeast-free diets. It was found that a vitamin mixture was essential for growth and development of the olive fruit fly in the yeast-free diet. None of the vitamin mixtures gave results equivalent to the yeast containing control diet. The first vitamin mixture did not give any improvement, the second one gave a considerable improvement and the third one improved further all parameters studied with the exception of larval survival. The best performance was obtained at the level of 0.50 g per 55 ml water and it could be used in future nutrirional work with the olive fruit fly.

1-Introduction

The importance of vitamins in insect nutrition has been known for a long time (Fraenkel and Blewett, 1943) and it has been reviewed by many investigators most recently by Hagen et al. (1984). Larval diets used for tephritidae, including the diets for the olive fruit fly *Dacus oleae* (Diptera: Tephritidae), contain yeast (Singh, 1977).

It is well documented that brewer's yeast contains almost all essential nutrients and it is very good source of certain vitamins. Its nutritional role for the olive fruit fly has been reported in diets with and without soy hydrolysate (Manoukas, 1975a). Omission of brewer's yeast from the larval diets resulted in severe reduction of pupal yield (Manoukas, 1977). Ideally, all nutritional and other dietary factors should be considered in order to substantiate the effects of brewer's yeast. However, based on the vitamin composition of brewer's yeast the hypothesis can be made that yeast-free diets may be deficient, among other nutrients in certain vitamins. Therefore, the present work was designed to test this hypothesis and to evaluate the effect of three commercially available vitamin mixtures on the performance of olive fruit fly larvae grown in a control and in a yeast-free diet.

2-Materials and methods

Eggs 48±6 h old were obtained from stock T of *D. oleae* flies maintained in the "DEMOKRITOS" laboratory for over twenty years at approximately 25° C, 65% R.H. and 14 h daily photoperiod according to a rearing technique described by Tsitsipis (1977). The experimental diets were prepared by adding the vitamin mixtures to a control diet (Tzanakakis et al., 1970, Manoukas, 1975) from which brewer's yeast was excluded and sucrose added. This diet for convenience is called yeast-free (YF) diet. The composition of the control diet and the YF diet is presented in Table I, together with their proximate analysis.

Table I-Composition of the control and yeast-free diet (YF).

Ingredient	Control	YF
Water, ml	55.00	55.00
Brewer's yeast (Schwechat, Australia), g	7.50	0.00
Soy hydrolysate enzymatic (N.B. Co. U.S.A.), g	3.0	3.00
Olive oil (Minerva Co., Greece), ml	2.00	2.00
Sucrose (Sugar Co., Greece), g	2.00	2.50[1]
Tween 80 (Merck AG, W. Germany), ml	0.75	0.75
K-sorbate (Merck AG, W. Germany), g	0.05	0.05
Nipagin (Merck AG, W. Germany), g	0.20	0.20
HCl, 2N, ml	3.00	2.00
Cellulose (Schleicher-Schull, W. Germany), g	26.50	26.50
Proximate analysis, % (based on ingredient content)		
Moisture	59.00	62.00
Protein (total nitrogen X 6.25)[1]	5.7	1.8
Lipids	2.3	2.3
Ash	0.8	0.2
Fiber	27.8	30.0
Nitrogen free extract (from 100)	4.4	3.7

[1] Increased by 0.50 g/55 ml water which represents approximately the quantity of sucrose or dextrose supplied by the addition of vitamin mixtures.

Final pH of all diets was 4.0±0.1. The vitamin mixtures were bought from ICN Biochemical Inc. USA under the name Vanderzant for insects (V), Diet Fortification (DF) and AIN No. 76 (AIN). Each vitamin mixture was added to the diet prior to the addition of soy hydrolysate, in the standard mixing procedure (Tzanakakis et al., 1970). The carrier (dextrose for V and DF mix and sucrose for AIN mix) and the vitamin content of each mixture given by the manufacturer is presented in Table II

Forty-five g of diet were placed in each container (replicate) and four replicates per treatment were used with 8 eggs/g diet. Description of containers, placement of eggs and hatchability checking is reported elsewhere (Manoukas, 1981). Number of larvae in the diet and their weight was recorded in a 2 g sample obtained randomly from each replicate after thorough mixing on the 8th day following hatching of the eggs. When more than 8% water was lost from a diet, distilled water was added to restore moisture level to near the initial level. Pupae were collected on the 12th day and every 2nd day thereafter. The days at which 90% of pupae had been collected was considered as "days to 90% pupation". Pupal weight was taken at least three days after pupation. Adult emergence was also recorded. Stastistical procedures employed were those described by Steele and Torrie (1960).

Table II- Vitamin composition of three commercial mixtures (mg/g)

VITAMIN	V[1]	DF[1]	AIN[1]
Vit. A (500,000 IU/g)	-	1.8	-
Vit. A (250,000 IU/g)	-	-	1.6
Vit. D (850,000 IU/g)	-	0.125	-
Vit. D (400,000 IU/g)	-	-	0.25
Vit. E (α-tocoph. eq.)	8	22	-
Vit. E (250 IU/g premix)	-	-	20
Vit. K (menadione)	-	2.2	-
Vit. K (menaquinone)	-	-	0.005
Vit. B_1 Thiamine	0.25	1	0.6
Vit. B_2 Riboflavin	0.5	1	0.6
Vit. B_6 Pyridoxine	0.25	1	0.7
Pantothenic acid	1	3	1.6
Nicotinic acid	1	4.25	3
Biotin	0.02	0.02	0.02
Folic acid	0.25	0.09	0.2
Vit. B_{12} (Trit. in mannitol)	2	-	-
Vit. B_{12}	-	0.01	0.01
Choline C_1	50	75	-
Vit. C, ascorbic acid	270	45	-
Inositol	20	5	-
P-amino benzoic acid	-	5	-
Carrier (to 1g)	Dextrose	Dextrose	Sucrose

[1] V, DF and AIN stands for Vanderzant, vitamin Diet Fortification mix and AIN 76 , respectiveley formulated and sold by ICN-Nutritional Biochemicals Co., U.S.A. (Catalog 1987-88).

3-Results and Discussion

Hatchability figures are not reported as they were satisfactory and did not differ among treatments. Each vitamin mix was tested at the level of 0.50 g/55 ml water upon larval performance, both in the control and in the yeast-free diet. The results are presented in Table III. The supplemented control diet gave results equivalent to unsupplemented control diets. It is suggested that the vitamin mixtures added had neither beneficial nor detrimental effects upon growth and survival of larvae at the larval density used. The YF diet depressed larval survival and growth and gave no pupae. The control diet supplemented with AIN mixture gave consideraably higher pupal yield and it took two days less to "90 % pupation" compared to the control but these differences failed to be of significance. This is in agreement with most other tephritids reared in yeast containing diets to which no vitamin supplementation is recommended (Singh, 1977). This is contrary to the recommended supplementation of yeast containing diets with Diet Fortification vitamin mixture for the cherry fruit fly larvae (Haish, 1975) and Vanderzand vitamin mixture for the olive fruit fly (Tischlinger, 1975).
These contradictions could be attributed to inadequate quality control of ingredients for tephritid larvae and lack of nutritional requirements for them.

Table III-Performance of the olive fruit fly larvae, reared in control and yeast-free diet supplemented with vitamin mixture, Vanderzant (V), Diet Fortification (DF) and AIN at 0.50 g/55 ml dietary water.

Diet[1]	Larvae /g diet	mg/lar-vae	pupae/ g diet	mg/ pupa	pupa-tion days	adults % pupae
Control (C)	6.2^{b}	4.9^{d}	4.8^{c}	6.1^{c}	14^{a}	83^{c}
Yeast-Free (YF)	1.9^{a}	0.1^{a}	0	-	-	-
C+V	5.8^{b}	5.1^{d}	4.7^{c}	5.8^{c}	14^{a}	81^{c}
C+DF	5.9^{b}	5.2^{d}	5.1^{c}	5.7^{c}	14^{a}	80^{c}
C+AIN	6.1^{b}	5.2^{d}	5.5^{c}	6.4^{c}	12^{a}	78^{c}
YF+V	1.4^{a}	0.1^{a}	0	-	-	-
YF+DF	5.3^{b}	$o.4^{b}$	1.0^{a}	2.5^{a}	26^{b}	24^{a}
YF+AIN	6.9^{b}	0.7^{c}	1.8^{b}	3.3^{b}	22^{b}	49^{b}

[1] Means in the same column followed by the same letter do not differ significantly at the 0.05 level of propability.

The YF diet supplemented with V mixture did not give any pupae, while the same diet with DF and AIN mixture gave pupae and adults. In addition AIN mixture gave satisfactory higher values of larval survival to pupation, larval weight, pupal weight and adult emergence compared to DF mixtures. The results of the experiments reported here are in line with those reported with the melon fly according to which a vitamin mixture was absolutely essential when larvae were grown in a synthetic medium without yeast (Srivastava et al., 1977) or with 0.5 g/100 ml diet (Chawla, 1966).

4-Conclusions

Over all consideration of the results presented may lead to the conclusion that vitamin supplementation is required when yeast-free diets are employed for rearing the olive fruit fly larvae. Of course it is not known which vitamins and at what levels are required by the larvae and why the Vanderzant mixture did not improve larval performance of the YF diet. It is safe to assume that the difference among the three mixtures was due to the wide differences in the vitamin composition (Table II). On the other hand since the YF diet differs in many nutritional and physical factors compared to the control (Table I) further work is required to understand the dietary requirements of the olive fruit fly and other tephritids.

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DEPRESSION OF EGG PRODUCTION IN *DACUS OLEAE* USING A PEROXIDASE INHIBITOR

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Summary

The peroxidase constitutes a major structural component of the chorions and it is responsible for the hardening process at the end of oogenesis (stage 14A), (Margaritis L.H., 1985a and Keramaris K.E.,et al,1991). The peroxidase action can be inhibited by the use of phloroglucinol during in vitro development of the follicles, (Mindrinos M., 1980).

This inhibitor is a phenolic substance (1,3,5 trihydroxy-benzene) and it is derived from plants. The plants and bacteria contain specific enzymes for the phloroglucinol metabolism, (reductaces, isomerases, oxidases), (Brune A. and Schink B., 1990, Haddock J.D. and Ferry J.G., 1989, Krumholz L.R. and Bryant M.P., 1988). Phloroglucinol has cytotoxic activity and geno-toxicity effects (Stich H.F.,et al, 1981).

We used phloroglucinol in *Dacus oleae* food at a concentration of 1mM, 10mM, 25mM, 50mM, 100mM and 400mM. At concentrations above 10mM egg-laying is inhibited due to defective eggshell hardening.

Also, the use of high concentrations of phloroglucinol during the in vitro development of the follicles affects protein synthesis by an unknown mechanism.

Further experiments are in progress in order to clarify the above points and practically utilize the results.

1 - Introduction

The insect eggshell or chorion is an extracellular structure serving several functions including respiration of the embryo and its protection from environmental hazards. The follicular epithelial cells surrounding the oocyte are responsible for the formation of the five major egg-shell layers: Vitelline membrane, waxy layer, innermost chorionic layer (ICL), endochorion and exochorion (Margaritis, L.H., 1985a and Margaritis, L.H., 1985b).

The endochorion and the ICL in *Dacus oleae* consist of several proteins synthesized according to a temporal and spatial developmental program (Mouzaki D.G. et al, 1991). These proteins crosslinked via di- and tri-tyrosine bonds, provide the eggshell with the required hardness and elasticity. In *Drosophila melanogaster* a peroxidase is responsible for the hardening process (Mindrinos M.N. et al, 1980, Margaritis L.H. 1985c). The enzyme that is also a functional and structural component of the chorion is activated by hydrogen peroxide secreted by the follicle cells at the last stage of oogenesis (Margaritis L.H., 1985c and Keramaris K.E. et al, 1991).

Phloroglucinol (1,3,5 -trihydroxybenzene) is a natural phenolic substance that inhibits the peroxidase activity and has been used for peroxidase inhibition during in vitro development of follicles (Mindrinos M.N. et al, 1980).

Phloroglucinol is produced from the degradation of plant polyphenols. It has been found that it extends the larval developmental period and increases the mortality of *Heliothis armigera* (Ananthakrishanan T.N., et al, 1990). It also inhibits the oxidative conversion of dopa to dopachrome under biomimetic conditions, a key step in the biosynthesis of melananin pigments (Crescenzi, O., et al, 1991).

Phloroclucinol in combination with plant hormones was found to inhibit in vitro rooting of microcuttings (Amin M.N. and Jaiswal V.S., 1989, and Poniedzialek, W. et al, 1986).

It is a simple phenol that causes in vitro carcinogenesis in CHO (Chinese Hamster Ovaries) and in combination with metals (Mg2+, Mn2+, etc.), induces chromatid breaks and exchanges (Stich H.F.,et al, 1981).

In addition, it is capable of reducing rectosigmoid motor in patients response after a test meal. This could explain its activity in abdominal pain associated with irritable bowel syndrome (Cargill, G., et al, 1989).

The metabolic pathways that lead to phloroglucinol degradation in Bacteria and plants have been studied. The NADPH dependent phloroglucinol reductase in *Eubacterium oxidoreducens* (Propionibacteriaceae) participates to the anaerobic catabolism of trihydroxybenzenes (Haddock J.D. and Ferry J.G., 1989). It has been found also that phloroglucinol-pyrrogaloll isomerase (Brune A. & Schink B., 1990 and Krumholz L.R. & Bryand M.P., 1986) and phloroglucinol reductase exist in fungi (Patel T.R., et al, 1990).

Finally, phloroglucinol oxidase has been extracted from tea leaves (Pruidze G.N., et al, 1976) and a peroxidase with phloroclucinol oxidase activity has been found in soybean (Toiguchi S., et al, 1989).

The present work is part of our research on phloroglucinol action during in vitro and in vivo follicle development of the olive fruit fly. Our attempt is to determine the minimum inhibitor concentration in the diet that could suppress the egg oviposition in *Dacus (Bactrocera) oleae* (Keramaris K.E. et al, in preparation). Propably, this substance could be used for biological pest control.

2 - Materials and Methods

In vivo effect of phloroglucinol

Phloroglucinol was dissolved in the liquid insect food (diet) at concentrations 0, 1, 10, 25, 50, 100 and 400 mM. Six pairs of ten days old and six pairs of newly emerged flies for each concentration were placed in individual cells and every second day the fecundity and the egg hatchability were measured. Experimental conditions and methodology have been reported elsewhere (Tsiropoulos G.J., 1985).

In vivo effect of phloroglucinol

Early choriogenic (stage 11A) follicles isolated from the ovaries after dissection, were incubated for four hours in Robb's tissue culture medium in the presence of ^{3}H-proline (Petri W.H. et al, 1979 and Robb J.A., 1969) and phloroglucinol concentrations 0.2 mM, 10 mM, 20 mM and 40 mM.

Incubation was followed by dissolving the follicles in STG (2% SDS, 50 mM Tris-HCl, 20% glycerol, pH 6.8) and SDS electrophoresis (Laemmli U.K., 1970), with linear gradient 10-15 % PAGE. The gel was incubated in PPO-DMSO, followed by exposure to X -ray film in -80 °C.

3 - Results

Effect of phloroglucinol in vivo.

The use of phloroglucinol in the insect's food at various concentrations, has been estimated based on the fact that it is a natural substance which can be metabolized from bacteria and plants and acts as a peroxidase inhibitor, without any effect to insect's survival but it might influence just the follicle system.

At concentration of 1mM in the diet, phloroglucinol did not affect any of the parameters studied. Higher concentrations reduced and finally suppressed fecundity. Fecundity reduction commences one day after the first meal and nullifies at the third day (diagram 1).

Flies that have been fed with phloroglucinol, at concentrations higher than 25 mM, from the beginning of their life, never oviposited eggs. At 10 mM the fecundity decreased gradually (diagram 2).

The effect of phloroglucinol after only one meal lasts for 1-2 days and after this, fecundity returns to normal levels (diagram 3).

During this experiment insect's behaviour was normal and the females were trying to oviposit but the eggs were dissolving and appeared as an amorphous mass. This is probably due to the fact that phloroglucinol prevented the chorion hardening so it couldn't resist the high pressures during egg passage through the narrow ovipositor.

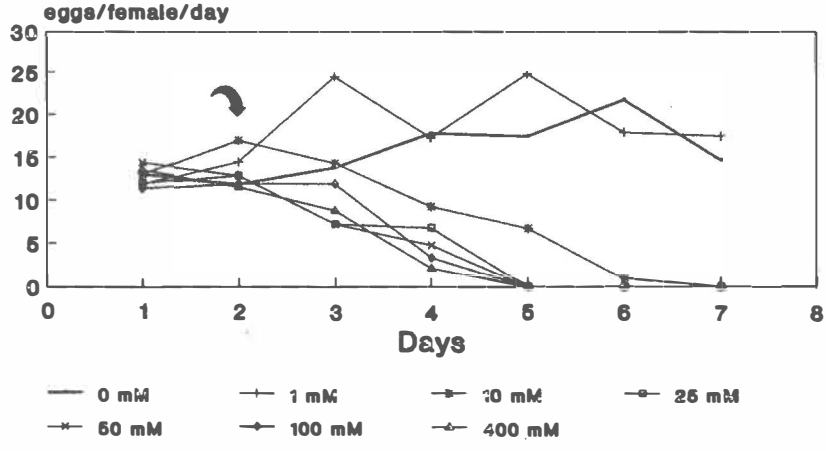

Diagram 1) Phloroglucinol effect upon insect's fecundity. The inhibitor was added in the diet at concentrations of 0mM, 1mM, 10mM, 25mM, 50mM, 100mM and 400mM. Flies digest phloroglucinol at the 10th day of their life. At 10mM, fecundity nullifies after the 6th day while at higher concentrations after the 5th day.

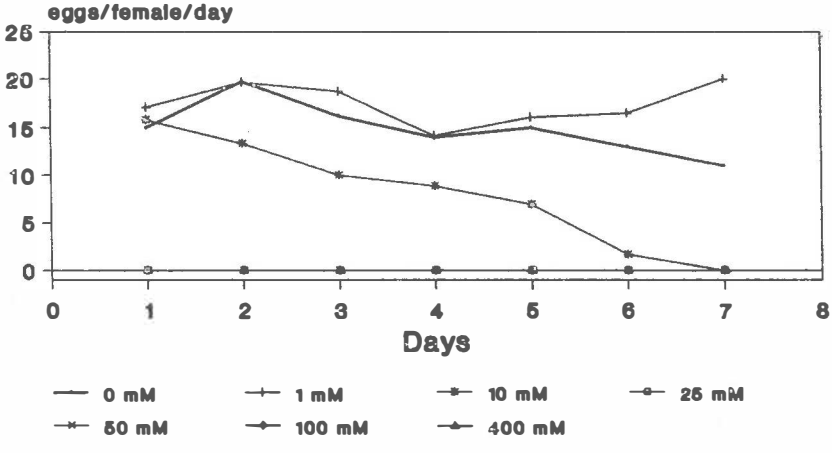

Diagram 2) Phloroglucinol effect upon insect's fecundity after feeding with various concentrations in the diet 0mM, 1mM, 10mM, 25mM, 50mM, 100mM and 400mM from the first day of their life. At 10mM, fecundity nullifies after the 6th day, while at the rest concentration there is no egg-laying.

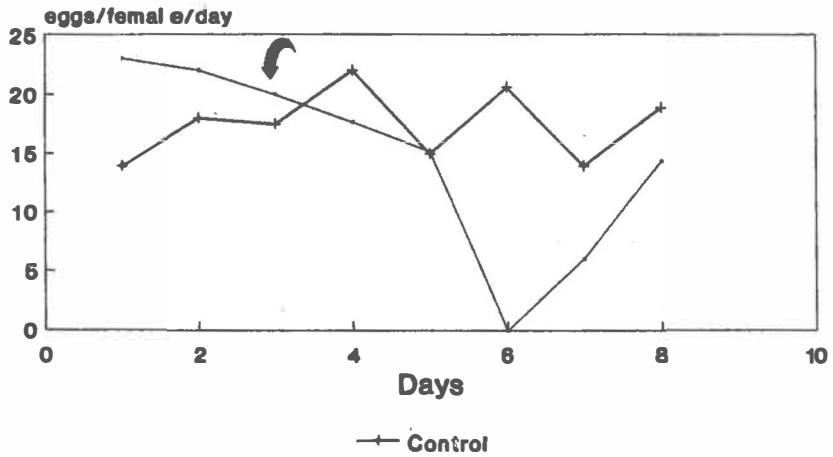

Diagram 3) Phloroglucinol effect upon insect's fecundity, when the inhibitor is used in diet alternately with normal food.
The flies feed on phloroglucinol (100mM) at the 10th day of development and only for one day. We observe the decrease of egg-laying up to zero within two days. The egg-laying rate return in normal level after two days.

Effect of phloroglucinol in vitro.

The toxic effects of phloroglucinol and other phenolic substances that have been reported, have led us to investigate its effect to the cellular model system of choriogenesis of *D. oleae*.

Fig.1) SDS-PAGE of whole follicle on 10-15% linear gradient after in vitro cultured follicles in Robb's medium with ^{3}H-Pro and phloroclucinol at concentration **a)** 0.2mM, **b)** 10mM, **c)** 20mM, **d)** 40mM. The phloroglucinol inhibits the protein-synthesis with unknown mechanism.

a b c d

After in vitro incubation of follicles in presence of ^{3}H-proline and various concentrations of phloroglucinol we concluded that in concentrations higher than 10 mM in the medium, the inhibitor suppress the protein-synthesis (Fig 1). It is not known yet if our substance affects the translational or transcriptional level.

4 - Conclusions

The use of phloroglucinol inhibits the action of the egg-shell peroxidase thus preventing the hardening process through the inability to form di-and try-tyrosine crosslinks. Suitable non-toxic concentrations inhibit fecundity reversibly due to breakage of of the eggs upon laying since the eggshell is no more elastic and cannot accomodate egg-passage through the narrow ovipositor.These conclusions may serve as the basis for pest control applications according to previous studies by Economopoulos (1989), Haniotakis & Skyrianos (1981) and Zervas (1986).

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Session 2

Biotechnical aspects of management of fruit flies populations

Chairman: D. M. HABIB
Secretaries: L. SANTOS
S. QUILICI

Report on Session 2

D. M. HABIB; L. SANTOS; S. QUILICI

Au cours de ce Meeting nous avons eu l'occasion d'assister et de discuter les résultats obtenus de recherches menées ces dernières années dans les régions circum-méditérranéennes, sur les mouches des fruits d'intérêt économique et particulièrement la mouche de l'olive *Bactrocera (Dacus) oleae* et la mouche méditerranéenne des fruits *Ceratitis capitata*.

Des communications présentées dans le thème "Aspects biotechniques de la gestion des populations des mouches des fruits ressort les points suivants:

L'utilisation des pièges jaunes englués appâtés séparément de trimédlure et d'hydrolysat de protéine permet une meilleure capture de la cératite que quand les deux attractifs sont placés ensemble, et peuvent être utilisés seulement pour la prédiction des infestations de la cératite à cause de leur effet négatif à l'égard des auxiliaires. Par contre dans le cas de la mouche de l'olive, les pièges Macphail et Rebell appâtés de sulfate d'ammoniaque semblent donner des résultats variant selon la saison en raison vraisemblablement de l'instabilité du sulfate d'ammoniaque, auquel il faut ajouter du Borax pour éviter son imprégnation rapide d'eau si l'humidité relative de l'air est importante.

Sur le plan pratique il convient de standardiser les modèles de pièges et songer à l'utilisation des pièges secs qui semblent être beaucoup plus pratiques.

Quant à la phéromone sexuelle de *Bactrocera oleae*, présente une dose optimale d'utilisation allant jusqu'à 10 mg / capsule, au delà de laquelle l'évaporation du produit augmente et l'efficacité de la capture diminue notablement (confusion sexuelle). Par contre la dose de la paraphéromone de la cératitie de 10 mg par capsule reste très attractive aux mâles.

En conclusion il y a lieu de standardiser et d'élaborer un modèle de piège pour chaque espèce en vue d'avoir d'une part un seul système de contrôle standard surtout si on envisage l'utilisation des pièges comme moyen d'avertissement ou de lutte et ou l'utilisation de la technique des mâles stériles et d'autre part favoriser l'expérimentation sur les phéromones de marquage qui ont donné des résultats satisfaisants chez *Rhagoletis cerasi* où des traitements même de la moitié des arbes utilisant ces substances, permettent une réduction notable des infestations. Par contre dans le cas de la cératite, les résultats sont parfois contradictoires, on a donc pu observer plusieurs piqûres de la mouche sur le même fruit, et même plusieurs pontes dans la même piqûre, ce qui fait que dans certains cas la phéromone de marquage de la cératite sécrétée par le tube digestif, ne joue pas souvent un rôle répulsif comme c'est cas des autres mouches de fruits.

En somme, plusieurs suggestions ont été proposées, portant sur l'organisation de programmes de recherche d'intérêt commun notamment la standardisation et la calibration des pièges ainsi que la poursuite des travaux sur les phéromones de marquage et les systèmes de contrôle et d'élargir davantage les connaissances écologiques sur la cératite et surtout l'effet des basses températures sur son développement pour pouvoir contrecarrer ce fléau.

ATRACÇÃO COMPARATIVA DE ARMADILHAS AMARELAS COM FEROMONAS NORMAIS OU DE LONGA DURAÇÃO PARA A *BACTROCERA (DACULUS) OLEAE* (GMELIN)

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SUMÁRIO

Ensaios com armadilhas amarelas a que se juntaram feromonas normais ou de longa duração para a *Bactrocera (Daculus) oleae* foram colocadas num olival da cv. "Blanqueta" na província do Alentejo (Portugal). Esta região está incluída na área fitoclimática iberomediterrânica caracterizada com altas temperaturas durante o verão e humidade relativa baixa, responsável em alguns anos por infestações baixas, inferiores aos niveis económicos de ataque.

Resultados de 1991 e 1992 são discutidos comparando a atracção das diferentes feromonas e a sua eficácia periódica.

SUMMARY

Trials with yellow traps added with standard pheromones or long life pheromone dispensers for *Bactrocera (Daculus) oleae* were estabelished on a olive grove of the cv. "Blanqueta" in the Alentejo province (Portugal). This region is included in the phytoclimatic iberomediterranean area characterized with high temperatures during the summer time and a low relative humidity responsible in some years for the low fruit infestations under economic injury levels.

Results from 1991 and 1992 are discussed comparing attractiveness of the different pheromones and the periodical effectiveness.

1 - INTRODUÇÃO

O projecto planeado de protecção integrada da oliveira incluí o estudo das pragas de maior importância económica para a região interior do Alentejo (Portugal). Entre estas, destaca-se a *Bactrocera (Daculus) oleae* (Gmelin) *(Diptera:Tephritidae)* como causadora de prejuízos muito significativos e directos sobre a produção, sob o seu aspecto quantitativo e qualitativo.

Contudo, apesar dos numerosos trabalhos já produzidos, não só em Portugal como, principalmente, em outros países mediterrânicos, a aplicação dos meios de luta, por parte dos olivicultores daquela região, têm-se baseado nas Recomendações (10) dependentes de um processo de contagens directas sobre amostragens de frutos colhidos ao longo do seu processo de desenvolvimento ou por um Sistema de Avisos emitidos por iniciativa de Serviços Oficiais Regionais.

Qualquer destes processos, para a maioria dos casos, por atrazo no conhecimento da informação sobre o nível potencial de infestação existente, no olival, pode fazer perder a melhor oportunidade

de intervenção para tratamentos, levando a que grande parte dos olivicultores, da região, tomem a iniciativa da aplicação de medidas profilácticas com caracter preventivo, por vezes, supérfluas por desnecessárias, outras vezes fora do momento que proporcionária maior eficácia.

Tentativas várias para conhecimento antecipado dos níveis de população de *B. oleae*, em determinado olival, evoluíram desde o uso de garrafas mosqueiras McPhail até às armadilhas amarelas adesivas de vários tipos com o objectivo de proporcionar resposta oportuna e mais correcta para actuação. Desde Economopoulos (3) utilizando armadilhas amarelas fluorescentes, passando pelos trabalhos coordenados por Boller, em 1978, nos quais Portugal prestou colaboração (4) e complementados com os de Agee (1), adoptou-se uma determinada coloração de amarelo mais atractiva para a mosca-da-azeitona.

Ensaios realizados na zona interior do Alentejo, durante 1979 e 1980, tiveram como objectivo a comparação entre capturas de *B. oleae* por meio de armadilhas McPhail, por acção quimiotrópica de uma solução de fosfato de amónio a 2%, ou utilizando Rebell constituídas por 2 placas amarelas adesivas colocadas em posição ortogonal de actuação, apenas, cromáticas e em confronto com a técnica de sondagem por "Knock-down" aplicando mevinfos à semelhança de outros trabalhos realizados por Neunschwander e Michelakis (8) na ilha de Creta em 1976 a 1978.

A melhor resposta, no período crítico a partir do início do desenvolvimento larvar nos frutos, foi conseguida com a utilização das armadilhas Rebell ao passo que as armadilhas McPhail apresentavam maior actratividade, sobre a mosca-da-azeitona logo a partir da primavera induzindo a que se tentasse melhorar, umas ou outras, através da adição de vários atractivos, inclusivé, do campo das feromonas específicas.

2 - MATERIAL E MÉTODOS

A Agrisense (U.K.) como entidade ligada à bioquímica na produção, também, de feromonas com acção sobre algumas pragas da oliveira, inclusivé, para a *B. oleae*, tem tentado, não só, melhorar as suas formulações de compostos atractivos como, ainda, quanto aos difusores dos respectivos vapores.

Algumas destas cápsulas foram já ensaiadas em Portugal mas, em 1991, por proposta da Agrisense em consonância com o projecto ECLAIR 209, o Departamento de Entomologia da Estação Agronómica Nacional e o Departamento de Olivicultura da Estação Nacional de Fruticultura Vieira Natividade realizaram novos ensaios experimentais comparativos entre feromonas de longa duração e outras consideradas normais colocadas sobre simples placas amarelas adesivas. Estas armadilhas para cada tipo de feromona foram mantidas em acção, mais de 6 meses, desde os primeiros dias de Junho até à colheita ou substituídas a intervalos de 4 semanas e colocadas em olival da cv. "Blanqueta". O ensaio compreendia, também, armadilhas amarelas adesivas simples, isto é, sem qualquer outro produto atractivo complementar.

Em outros olivais próximos colocaram-se armadilhas McPhail com solução de fosfato de amónio a 2% para observação laboratorial das

fêmeas capturadas quanto à evolução e maturação dos ovários, além de servirem para o traçado da respectiva curva-de-voo para comparação com outras.

Todos os ensaios compreendiam esquemas de implantação em 4 repetições para qualquer tipo de armadilha ficando estas afastadas, pelo menos, 35 metros umas das outras.

As leituras das capturas de *B. oleae*, para análise, foram agrupadas em totais semanais.

Complementarmente, em árvores próximas do mesmo olival eram retiradas pequenas amostras da frutificação para observação laboratorial para detecção de infestações com registo de picadas ou manchas na epiderme dos frutos provocadas por acção, possível, de insectos, de larvas vivas ou mortas nos frutos, de galerias ou, ainda, orifícios de saída das larvas.

Os frutos caídos a partir do tamanho de um grão de pimenta, e até à colheita, eram contados e examinados laboratorialmente para a detecção possível das causas de sua queda prematura.

À colheita, e por cada árvore em ensaio foi retirada da produção colhida uma amostra de um quilo para exame em laboratório.

3 - CARACTERIZAÇÃO CLIMÁTICA DA REGIÃO

A região onde os ensaios foram implantados é classificada, sob o aspecto fitoclimático, como iberomediterrânea, caracterizada por um verão com altas temperaturas e humidade relativa com teores baixos. Pormenorizando, durante os meses de verão ocorrem muitos dias em que as temperaturas máximas diárias ultrapassam os 35°C ao mesmo tempo que a humidade relativa, no período mais quente, pode oscilar entre 35 e 50%. As consequências destes factos levaram a que Azevedo (2) incluísse a região considerada na zona II e que corresponderia a um comportamento da *B. oleae*, nas suas linhas gerais, a um acentuado atrazo das primeiras posturas viáveis acrescidas frequentemente nos níveis de população larvar por morte de muitas ainda no interior dos frutos.

Trabalhos já antes realizados por Fletcher et al. (5) sobre a inibição de posturas por parte da *B. oleae* em condições laboratoriais quanto a temperatura ambiente oscila entre 26 e 29°C e a humidade relativa é baixa oscilando a volta dos 45% foi confirmada na prática e na área de Elvas por Guerra (6) e, em cada ano, verificada em exames de laboratório quanto à oogénese e oviposição nos meses de verão.

Complementarmente, temperaturas máximas diárias superiores a 35°C verificadas em mais de 4 dias seguidos, associados a humidade relativa baixa, têm sido o ponto de referencia justificativo para a morte de muitas larvas resultantes de posturas viáveis mas em períodos críticos de verão, particularmente nos meses de Julho e Agosto.

Situações deste tipo originam flutuações na população de *B. oleae* muitas vezes abaixo dos níveis económicos de ataque.

4 - RESULTADOS

O objectivo principal que se pretende com as armadilhas é obter informação oportuna e tão correcta quanto possível sobre a curva-de-voo da *B. oleae* com amplitude suficiente para corresponder a níveis determinados de infestação da produção. A calibragem das mesmas, quanto a prejuízos, poderá proporcionar informações seguras quanto aos riscos na decisão para tratamentos.

A implantação do ensaio seguiu as recomendações da Agrisense e o traçado comparativo das curvas-de-voo encontra-se representado nos gráficos I (1991) e II (1992). Nestes gráficos separam-se os traçados correspondentes às armadilhas com feromonas de longa duração das carregadas com feromonas normais para proporcionar melhor análise.

A colocação das armadilhas realizou-se logo nos primeiros dias de Junho quando as oliveiras ainda se apresentavam nos estado fenológico I correspondendo a existencia de frutos vingados já com tamanho intermédio entre o grão de pimenta e o de ervilha terminando as contagens cerca de uma semana após a colheita, concretamente em fins de Novembro (24-25 Nov. 1991) e primeiros dias de Dezembro (4-6 de Dez. 1992). Complementarmente, também se utilizou uma armadilha amarela adesiva simples para confronto com ensaios realizados em 1979-80 (4).

Nos gráficos citados, além das referências às contagens semanais das capturas de adultos de *B. oleae* também figuram os valores da pluviosidade e a indicação do número de dias em que a temperatura máxima diária foi superior a 35ºC.

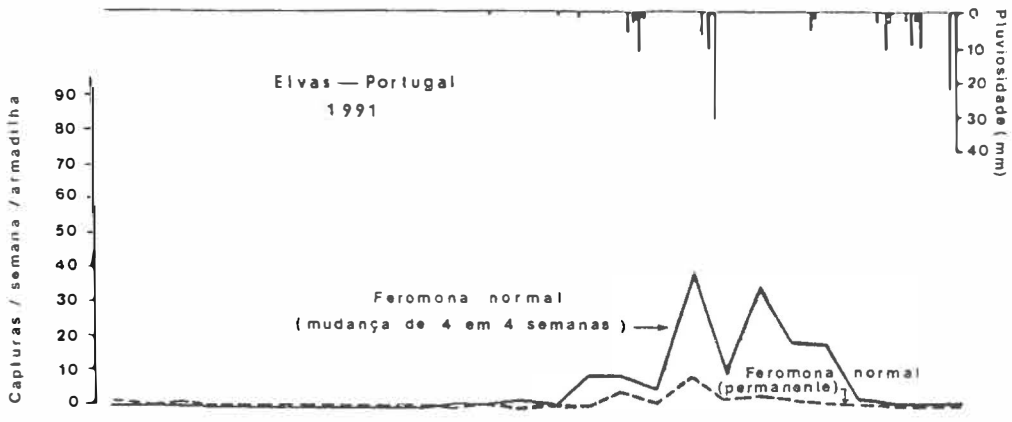
Tanto em 1991, como para 1992, as curvas-de-voo com maiores cotas de capturas corresponderam à utilização de armadilhas amarelas complementadas com feromonas de longa duração quando comparadas com idênticas armadilhas com feromonas "normais" e em igual tempo de actuação.

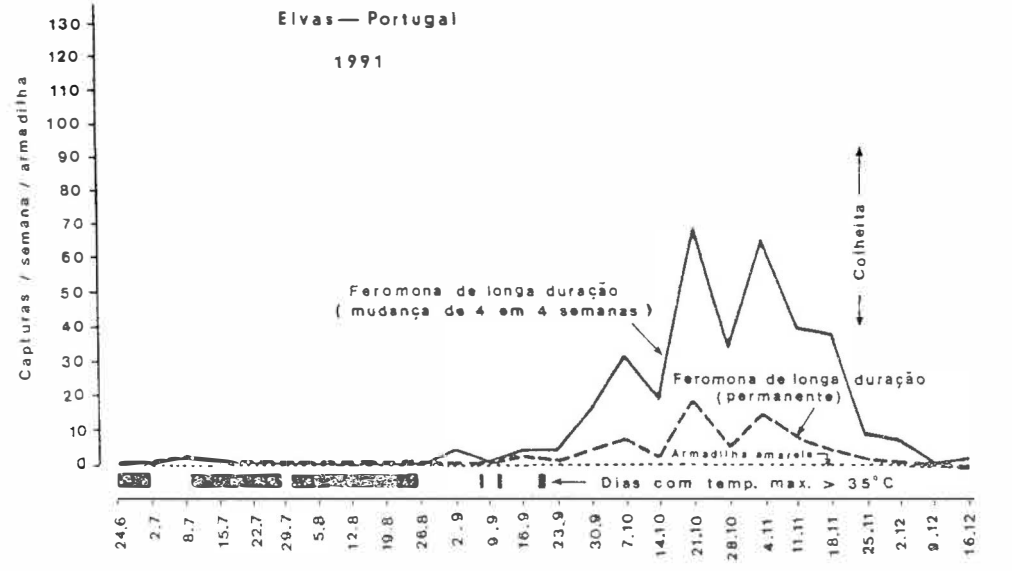
A relação sexual encontrada nas capturas foi nitidamente demonstrativa, para qualquer das feromonas utilizadas, que estas eram essencialmente de atracção masculina quando para a maioria dos totais semanais se verificava 100% de machos colhidos. Nos casos em que se registaram maior número de fêmeas capturadas estas correspondiam a 3,3% (em 1991) ou a 4,6% (em 1992) do total considerado, o que aconteceu no periodo de maior infestação, em meados de Outubro.

Comparando as curvas-de-voo de 1991 com as de 1992 verifica-se que:

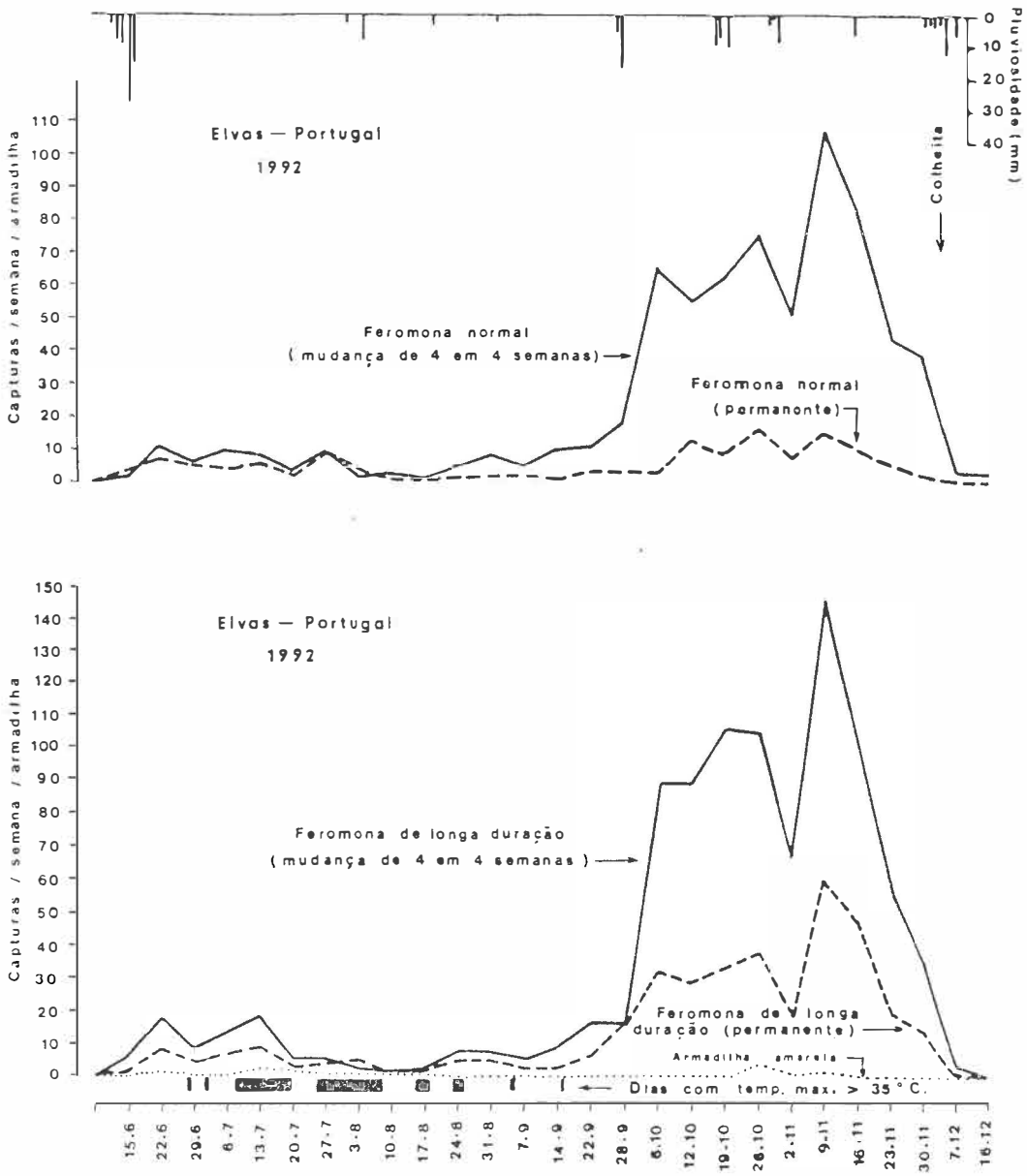
a) Em 1992 as cotas de capturas foram superiores cerca de 2 vezes às de 1991, em particular, no período de meados de Setembro a fins de Novembro revelando maior nível populacional infestante da mosca-da-azeitona;

b) A armadilha amarela simples não revelou qualquer poder atractivo, sobre a *B. oleae*, quando integrada ou, ainda que, suficientemente afastada da área de ensaio;





Curvas de voo de *Bactrocera (Dacus) oleae* (Gmelin) utilizando armadilhas amarelas simples, com feromonas normais e de longa duração (Agrisense).



Curvas de voo de *Bactrocera (Daculus) oleae* (Gmelin) utilizando armadilhas amarelas simples, com feromonas normais e de longa duração (Agrisense).

c) No período mais quente do verão e quando as temperaturas máximas diárias se prolongam, por alguns dias consecutivos, acima dos 35ºC a que corresponderão, também, temperaturas médias geralmente acima dos 26ºC a actividade de voo de disperção da *B. oleae* fica muito reduzida, quase nula em 1991.

A análise, para cada ano, revelou:

a) As curvas-de-voo com qualquer das armadilhas apresentam traçado muito semelhante, quase em pararelo umas com as outras excepto quanto a cotas de capturas;

b) As curvas de maior amplitude foram as que utilizaram as feromonas de longa duração com substituição das mesmas de 4 em 4 semanas;

c) As feromonas normais substituídas, também, a intervalos de 4 semanas proporcionaram uma curva um pouco mais reduzida (cerca de 25%) que a anterior;

d) As armadilhas com feromonas de longa duração em uso permanente, durante todo o ensaio, revelaram menor representatividade ainda mais evidente em 1991;

e) Com duração permanente as armadilhas com feromonas normais apresentam fraca representação das populações infestantes dos olivais, particularmente, no período de prematuração dos frutos.

5 - INFESTAÇÕES DE *B. oleae* NOS OLIVAIS DO ALENTEJO INTERIOR. NÍVEIS ECONÓMICOS DE ATAQUE.

Em relação às curvas-de-voo observadas haverá que as interpretar no sentido de se conhecerem os níveis económicos de ataque e a oportunidade de intervenção para evitar prejuízos.

Para tanto, a calibragem das armadilhas em relação aos estragos de que a *B. oleae* ocasiona na produção destinada à extracção de azeite terá que ser baseada nos seguintes pontos (7):

a) Perda provocada por queda permatura de frutos atacados no período antes da colheita;

b) Desgaste nos frutos com perda de polpa provocada pela actividade da larva no seu desenvolvimento;

c) Perda de qualidade do azeite extraído de frutos atacados pela *B. oleae.*

Esta última fracção de prejuízos sobre a produção conseguida, ainda que importante, não foi considerada nos ensaios anteriores por fazer parte de um processo tecnológico com base em metodologia própria.

O período de prematuração das azeitonas até à respectiva colheita terá que ser examinado atendendo a duas épocas distintas de ataques e susceptíveis de provocar prejuízos: o primeiro, ocorrendo

desde meados de Julho a princípios de Agosto e dependente da precocidade das cultivares com tentativas de oviposição (frutos atingindo cerca de 2/3 do peso final) resultando marcas na epiderme; o segundo, com início no período de Setembro-Outubro agora mais importante para a produção de azeite.

Considerando o número de gerações, verifica-se que a *B. oleae* passa o inverno no estado adulto, em actividade reduzida, abrigando-se nas fendas ou orifícios dos troncos, ou sob a forma de pupa, geralmente enterrada no solo. Uma geração preliminar às gerações que se seguirão, sobre a oliveira, atacando a produção do ano terá origem em infestações sobre frutos não colhidos e remanescentes sobre as árvores. Em princípio será de nível populacional relativamente baixo porque, além de dependente dessa frutificação da época anterior, será muito influênciada pelas condições climáticas estivais já referidas, antes, para a região do Alentejo.

A primeira geração, propriamente dita, resultará dos adultos então emergidos em Junho-Julho, (nitido nas curvas-de-voo de 1992) e que atacarão a nova frutificação mais precoce de fins de Julho a princípios de Agosto (com importância maior para a azeitona de conserva). A partir de fins de Agosto princípios de Setembro verifica-se nova emergência sucessiva de adultos para reinício de nova infestação dos olivais, considerada como de 2ª geração potêncialmente da maior importância pela oviposição continuada sobre a nova frutificação já desenvolvida.

Esta 2ª geração dará origem a uma nova 3ª geração que se irá sobrepondo à presença e actividade infestante da população anterior. Daqui resultará uma maior captura de adultos cujos picos na curva-de-voo não permitem distinguir as inflexões pertencentes a uma e outra geração.

Da 3ª geração resultarão, novamente, adultos hibernantes ou pupas enterradas no solo. Logo que as condições de temperatura de primavera se tornem favoráveis darão origem à já referida geração preliminar também designada como última da série dependente da mesma época de produção, neste caso, mais propriamente designada como 4ª geração originada em frutificação da época passada.

As decisões para tratamentos, preventivos ou curativos, dos ataques de *B. oleae* da mosca-da-azeitona serão tanto mais válidas (9) quanto mais as curvas-de-voo reflectirem os níveis de população existentes em cada olival e forem conhecidos os respectivos níveis económicos de ataque.

Enquanto que na análise directa da produção potencial e produção real, os índices e valores de infestação encontrados, em 1991, foram indicativos de estarem nítidamente abaixo dos níveis económicos de ataque, (1,6% da frutificação com ataques de *B. oleae* na queda prematura e 0,53% na produção colhida); para 1992, a situação não ficou bem definida (3,1% com ataques de *B. oleae* na queda antecipada e 6,2% à colheita) pelo fraco valor produtivo de algumas oliveiras com fracas disponibilidades hídricas, no solo, originando a queda fisiológica de frutificação a que se juntou a acção da *Prays oleae* provocando prejuízos acentuados.

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UTILIZAÇÃO EM PORTUGAL DE SISTEMAS DE ARMADILHAS NA LUTA CONTRA *CERATITIS CAPITATA* (WIED.) EM CITRINOS

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Abstract

The use of trapping systems in the management of *Ceratitis capitata* (Wied.) populations in portuguese citrus groves. Several trap models have been tested for studding *Ceratitis capitata* (Wied.) in citrus groves in the Algarve. An experiment was carried out using traps as a control method. This was compared with the following four modalities: a blank (without any treatment) and three different chemical interventions (strip treatments with dimethoate + hydrolyzed protein; according to the regional advisory system with dimethoate; and intensive treatments with dimethoate on a ten days periodicity).

In addition to the economical study of different control methods, as a basis for the decision making process, several aspects of the mediterranean fruit fly bioecology where analyzed: fruits on the ground, damaged fruits in the tree (localized at the canopy level and at the fruit label) and damaged fruits on the ground.

Resumo

Têm vindo a ser ensaiados diversos modelos de armadilhas para o estudo de *Ceratitis capitata* (Wied.) em citrinos no Algarve. Neste âmbito foi efectuado um ensaio utilizando armadilhas como meio de luta. Estas foram comparadas com as seguintes quatro modalidades: uma testemunha (sem tratamento) e três com intervenções químicas (tratamento em faixas com dimetoato + proteína hidrolizada; segundo o sistema de avisos tratamento com dimetoato; e tratamento intensivo com dimetoato e periodicidade de 10 dias).

Além de estudos de impacto económico dos diferentes meios de luta para as tomadas de decisão, tais como: frutos caidos; picados na árvore (com a localização da picada ao nível da copa e no fruto); picados entre os caídos. Foram também analisados vários aspectos de bioecologia da mosca do mediterrâneo.

1-Introdução

A mosca do mediterrâneo, *Ceratitis capitata* (Wied.), constitui praga chave de diversas fruteiras, nomeadamente em citrinos no Algarve, a principal região produtora (Carvalho, 1988; Carvalho & Fernandes, 1993).

As condições climáticas, aliadas à estrutura fundiária da exploração agricola, em geral constituída por pomares de pequena dimensão, do tipo familiar e com elevada diversidade de hospedeiros susceptíveis ao ataque de mosca do mediterrâneo, contribuem para o estatuto que esta praga tem.

Actualmente, na região, o combate contra *C. capitata* baseia-se exclusivamente na luta química, com os seus graves inconvenientes económicos, toxicológicos e ecotoxicológicos.

Para o desenvolvimento de estratégias de luta mais adequada, orientadas no sentido da Protecção Integrada, é fundamental ter os necessários conhecimentos sobre a dinâmica das

populações, para se poder actuar com as intervenções fitossanitárias na oportunidade mais aconselhável (Ros, 1988). Para o efeito, assume particular interesse a utilização de armadilhas (Delrio & Ortu, 1988; Cunningham, 1989; Economopoulos, 1989; Ros, 1990; Thomson, 1991).

O emprego de armadilhas tem a maior importância na monitorização e nos estudos de dinâmica populacional da mosca do mediterrâneo. Nas condições do Algarve foram testadas para estes efeitos diversos tipos de armadilhas. Com base nos resultados desses estudos foi averiguada a possibilidade do emprego de armadilhas como meio de luta contra *C. capitata*.

2-Material e métodos

O ensaio foi efectuado na zona de Tavira, num pomar industrial de laranjeira doce (*Citrus sinensis* Osbeck), variedade "Valencia late", no período de 9 de Junho a 28 de Agosto de 1993. Foram consideradas cinco modalidades, quatro das quais numa área de 3380 m^2, correspondendo a quinta modalidade a uma parcela de pomar onde os tratamentos foram efectuados pelo agricultor.

Cada modalidade abrangeu 25 árvores sendo o seu compasso de 5m x 4m.

As modalidades do ensaio foram as seguintes:

Modalidade A- Testemunha, na qual não foi efectuado qualquer tratamento.

Modalidade B- Tratamento em faixas no lado Sul da copa, num quinto da parcela, utilizando dimetoato + proteína hidrolizada. A decisão do tratamento foi tomada com base em estudos sobre a dinâmica populacional de *C. capitata* que decorreram num pomar próximo.

Foram efectuados três tratamentos: em 9 e 29 de Junho e 8 de Agosto.

Modalidade C- Armadilhas como meio de luta (Ortu & Prota, 1988). Utilizaram-se 21 placas cromotrópicas amarelas com proteína hidrolizada e quatro contentores de plástico transparente com trimedlure (uma armadilha por árvore).

•placas cromotrópicas amarelas com dimensões de 150mm por 200mm. Estas foram revestidas pela substância adesiva (Napvis), tendo-se adaptado uma tira dupla de papel mata-borrão com 90mm por 30mm, impregnada com proteína hidrolizada e colocada num dispositivo em telhado, fixo à aresta superior de cada placa (Carvalho & Pereira, 1993).

•contentor de plástico transparente cilindrico, com 80mm de altura e 53mm de diâmetro, com tampa de plástico opaco branco, na qual foi colocada pelo lado interior um difusor de algodão com 1ml de trimedlure. Na parte superior da parede do contentor, foram feitos seis furos circulares de 10mm de diâmetro, para permitir a difusão na atmosfera do odor atractivo e a entrada das moscas. Em cada contentor foram utilizados 150ml de água com gotas de detergente (Carvalho & Pereira, 1993).

As placas foram substituídas de 20 em 20 dias, com adição de proteína de 10 em 10 dias (Pereira & Carvalho, 1993). Aos contentores apenas se adicionou água com detergente de 10 em 10 dias, permanecendo o mesmo difusor ao longo do período de manutenção do ensaio.

Modalidade D- Tratamento de 10 em 10 dias com dimetoato, num total de oito tratamentos.

Modalidade E- Tratamentos efectuados pelo agricultor com base nos avisos emitidos pelos serviços oficiais.

Foram efectuados quatro tratamentos: em 11 e 25 de Junho, 20 de Julho e 19 de Agosto.

As observações foram efectuadas com periodicidade de 10 dias em nove árvores do interior da parcela de cada modalidade, registando-se:

•número de picadas por fruto, em 10 frutos por árvore localizados na periferia da copa. A partir da quinta observação foi registada a localização no fruto das picadas (dividiu-se o fruto em quatro zonas: a-externo inferior; b-externo superior; c-interno inferior; d-interno superior).

•número de frutos caídos.

•número de picadas por fruto caído, excluindo-se aqueles em que a avançada deterioração já não permitia a sua detecção. No caso em que o número de frutos foi superior a 100, a amostragem incidiu apenas neste último quantitativo.

•na modalidade C foi registado de 10 em 10 dias o número de adultos de *C. capitata*, coccinelideos e crisopideos, mas estes predadores com uma periodicidade de 20 dias.

•no final do ensaio foi efectuada a colheita de frutos nas árvores em observação, procedendo-se ao seu registo quantitativo e qualitativo.

3-Resultados e discussão

Na Fig. 1 encontra-se registada a evolução do número de frutos caídos por árvore em cada modalidade. Pela análise dos resultados, verifica-se que existe sempre um relativo paralelismo entre as modalidades, o que sugere não se dever atribuir exclusivamente ao ataque da mosca do mediterrâneo a queda dos frutos, sendo também admissível a influência de factores como a sobrematuração, estado fisiológico da planta, vento, problemas fitossanitários (destacando-se a altemariose) e outros.

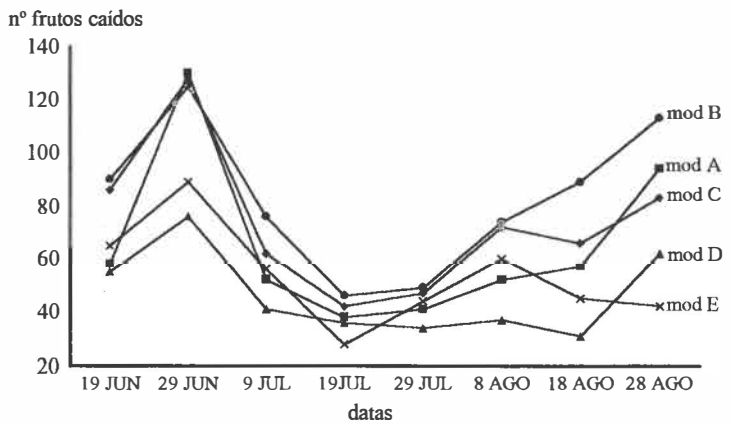

Fig. 1-Frutos caídos (em nove árvores por modalidade), ao longo do período de ensaio.

De referir, ainda, que o número de frutos caídos se relaciona com a produção de cada árvore.

A percentagem de frutos caídos, picados pelo insecto, constitui um dos parâmetros de avaliação da intensidade de ataque de *C. capitata*.

Os registos e a evolução da queda de frutos picados, encontram-se representados, respectivamente, no Quadro I e Fig. 2.

Os resultados permitem verificar que existem acentuadas semelhanças nas primeiras quatro observações das diferentes modalidades. A partir da segunda observação houve uma maior percentagem de frutos picados na modalidade A, sendo claramente superiores nas últimas quatro observações.

Quadro I-Valores, em percentagem, de frutos caídos picados ao longo do ensaio para as diferentes modalidades.

Mod.	19 Jun	29 Jun	9 Jul	19 Jul	29 Jul	8 Ago	18 Ago	28 Ago
A	48,1	47,0	51,1	48,6	53,7	46,0	46,4	36,3
B	40,3	39,0	40,3	42,1	39,5	36,6	25,6	23,0
C	48,0	45,0	51,1	46,9	22,7	34,3	25,8	25,1
D	51,4	44,6	37,5	44,4	33,3	30,0	20,8	22,6
E	51,1	39,2	46,9	30,8	39,5	28,6	27,9	27,5

De referir que os resultados nas modalidades tratadas (em especial na D), não foram tão reduzidos como seria de esperar. No entanto, ao longo do tempo, houve um decréscimo na percentagem de frutos caídos picados.

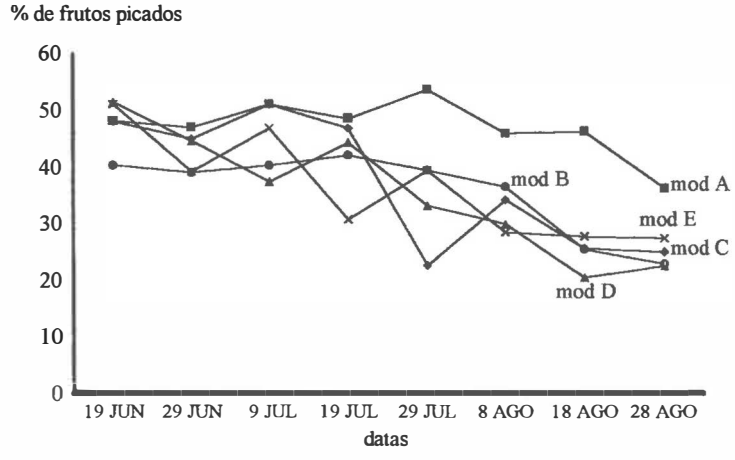

Fig. 2-Evolução, em percentagem, de frutos caídos picados ao longo do tempo para as diferentes modalidades.

Relativamente aos frutos na árvore picados (Quadro II), verifica-se que a modalidade A (testemunha) apresenta quase sempre uma intensidade de ataque superior ás outras modalidades (excepções na terceira e sétima observação).

Como seria de esperar, a modalidade D apresenta valores de frutos picados quase sempre inferiores ás restantes modalidades (excepto na segunda observação).

A percentagem de frutos na árvore picados é inferior à dos frutos caídos.

Embora dependendo da variedade e de outros factores (estado de maturação, estado fisiológico e condições ambientais), nos citrinos, normalmente, só em cerca de 0,5% dos frutos picados há êxito da postura com desenvolvimento das larvas e consequente queda de frutos (Bodenheimer, 1951). Todavia, alguns frutos não caiem em consequência directa da picada, mas sim do desenvolvimento de agentes secundários, tais como bactérias e fungos. Daí a explicação plausível do tão elevado

número de frutos picados caídos. Existem, no entanto, dados que contribuem para ajudar a esclarecer a influência do comportamento de *C. capitata* relativamente à queda dos frutos.

Quadro II-Número de frutos na árvore picados (em 90 frutos por modalidade), ao longo do tempo.

Mod.	19 Jun	29 Jun	9 Jul	19 Jul	29 Jul	8 Ago	18 Ago	28 Ago
A	10	37	26	28	21	17	15	16
B	8	25	15	19	12	13	12	11
C	5	14	13	24	10	9	9	8
D	2	20	13	18	10	7	4	4
E	9	16	33	17	14	8	16	9

Assim foram analisados 556 e 873 frutos atacados, respectivamente, na árvore e no chão, tendo-se calculado a frequência do número de picadas por fruto (Fig. 3). Este dado, de certo modo traduz a incidência dos ataques da mosca do mediterrâneo aos frutos, podendo repercutir-se, consoante a intensidade das picadas por fruto, na sua queda.

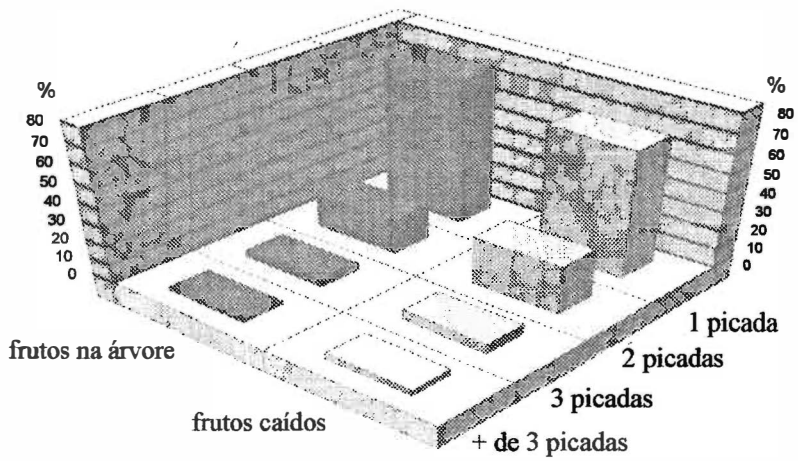

Fig. 3- Frequência de picadas (em percentagem) nos frutos atacados por *Ceratitis capitata* (na árvore e caídos).

Cerca de 95% e 92% dos frutos atacados, respectivamente, na árvore e no chão tinham apenas uma ou duas picadas. Na árvore, só 1,8% é que apresentavam mais de três picadas, enquanto que o correspondente valor nos frutos caídos foi de 3,0%. Estes resultados dão uma indicação, embora não muito evidente, de uma maior queda de frutos nos casos em que houve mais de uma picada.

Num ponto de vista do relacionamento das picadas com o comportamento das fêmeas, verifica-se que a localização daquelas obedece a uma determinada tendência. A partir da quinta observação, foi registada essa localização, num total de 294 frutos picados na árvore.

Definiram-se quatro zonas de picada no fruto, dividindo-o equatorialmente em parte superior e inferior e, por sua vez, em duas faces, exterior e interior (Fig. 4).

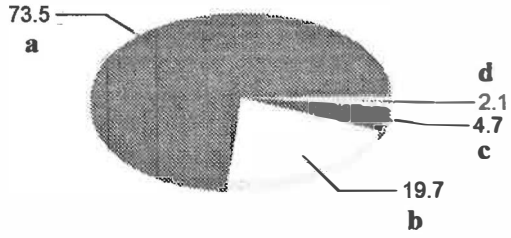

Fig. 4- Frequência (em percentagem) dos locais de picada de *Ceratitis capitata* no fruto na árvore: **a**-exterior inferior; **b**-exterior superior; **c**-interior inferior; **d**-interior inferior.

Os resultados das observações demonstram ter havido uma diferença muito acentuada de 93,2% e 6,8%, respectivamente, entre a zona de exposição do fruto exterior e o interior. Esta diferença pode ser explicada pelo efeito visual do fruto (através da sua reflectância) sobre as fêmeas quando estas aproximam da árvore (Prokopy & Owens, 1983).

Relativamente à face inferior e superior do fruto as diferenças também foram acentuadas, podendo ser devidas ao facto da zona inferior apresentar uma coloração mais sombreada, de forma a desencadear um tropismo positivo no insecto (Agee *et al.*, 1982).

Deu-se ainda atenção ao número de frutos picados na árvore por quadrante (Fig. 5). A exposição Sul foi a que correspondeu a um valor mais elevado (33,1%), seguido do quadrante Oeste (30,2%).

Estes resultados são coerentes com as estratégias geralmente seguidas na monitorização e na luta contra *C. capitata*. No primeiro caso, correspondem à opção de se escolher a exposição mais favorável para a captura de adultos com armadilhas. No segundo caso, justificam a aplicação no quadrante mais aconselhável (Sul) de insecticida em faixas, quando em combinação com um atractivo (normalmente proteína hidrolizada).

As observações descritas foram acompanhadas pelo estudo da evolução das capturas de *C. capitata* com armadilhas utilizadas na modalidade C (Fig. 6).

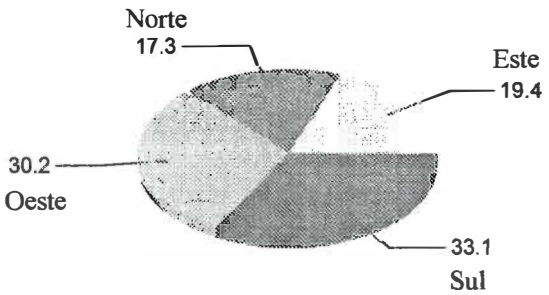

Fig. 5-Distribuição da frequência (em percentagem) de frutos picados na árvore segundo a exposição.

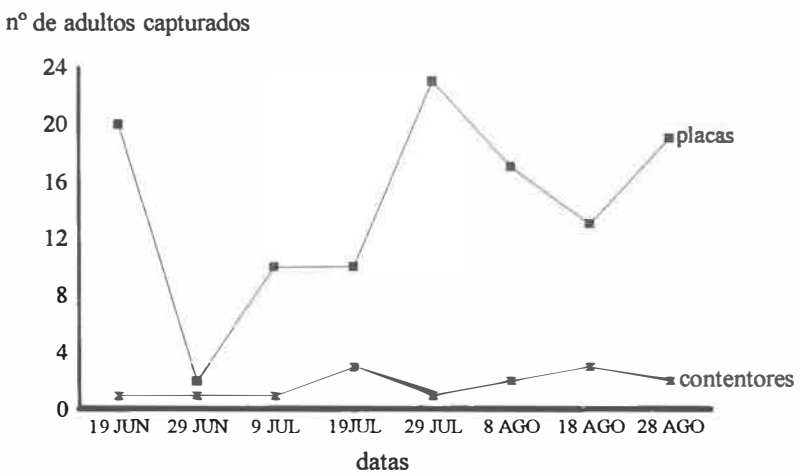

Fig. 6-Número de adultos de *Ceratitis capitata* capturados na modalidade C, em 4 contentores cilíndricos e 21 placas cromotrópicas amarelas.

Foram utilizados os dois tipos de armadilhas referidos com o objectivo de capturar machos (contentores cilindricos com trimedlure) e essencialmente fêmeas (placas cromotrópicas amarelas com proteína) de *C. capitata* (Delrio & Ortu, 1988; Carvalho & Pereira, 1993; Pereira & Carvalho, 1993).

Estes elementos revelam a presença constante, embora com uma abundância populacional oscilante, na parcela em estudo.

Na perspectiva da avaliação do impacto económico dos resultados do estudo realizado, fez-se a análise de custos dos frutos comercializáveis picados e do custo dos tratamentos.

No Quadro III reúnem-se os dados referentes à produção média das árvores em observação.

Quadro III-Produção média das árvores em observação.

Qualidade	Produção (kg)*	Frutos x kg^{-1}	Frutos
Comercializável	412	5,8	2389,6
Não comercializável	84	9,1	688,5

* dados referentes a nove árvores

A determinação do número de frutos x kg^{-1} foi obtida por amostragem de acordo com a aceitação comercial da produção baseada no tamanho dos frutos.

Com base nos dados do Quadro III determinou-se a frequência de frutos comercializáveis (**p**):

$$p = \frac{2389,6}{2389,6 + 688,5} = 0,776$$

A quantificação das perdas verificadas, em kg de frutos comercializáveis, calculou-se de acordo com a seguinte equação:

$$y = \frac{\sum_{i=1}^{8} (x_i \times c_i) \times p}{f}$$

Em que:

y-kg de frutos comercializáveis perdidos
xi-frequência de frutos caídos picados na observação *i*
ci-número de frutos caídos na observação *i*
p-frequência de frutos comercializáveis
f-número de frutos comercializáveis x kg^{-1}

No Quadro IV são apresentados os valores calculados de y e as correspondentes perdas, tendo em conta o preço real da comercialização dos frutos (45$00). Também se apresenta o valor das diferenças, entre a modalidade que apresentou maiores perdas (A) e as restantes modalidades.

Pela análise dos dados do Quadro IV, verifica-se que a modalidade D foi a que apresentou menores perdas, ficando as modalidades B e C próximas da testemunha.

Para a determinação do custo de tratamento, teve-se em consideração preços reais de mão de obra, aluguer do tractor devidamente equipado e com respectivo tractorista, água e produto fitofarmacêutico, excluindo-se por indeterminação os custos ecotoxicológicos. Destes, destaca-se o efeito sobre predadores e parasitóides.

Quadro IV-Valores de y (kg de frutos comercializáveis perdidos), perdas totais e diferença entre as perdas da testemunha em relação ás restantes modalidades.

Mod.	y (kg)	Perdas	Max. perdas- Mod. *j**
A	32,2	1449$00	-
B	30,4	1368$00	91$00
C	31,0	1395$00	54$00
D	18,3	823$50	625$50
E	21,6	972$00	477$00

* Mod. *J* = B, C, D e E

Os valores obtidos para o custo do tratamento com dimetoato foi 9990$00/ha. Para o tratamento em faixas com dimetoato + proteína hidrolizada, em 1/5 da área de pomar, foi 3445$00/ha. Estes valores reportados a nove árvores (180m^2), correspondem, respectivamente, a 180$00 e 62$00.

Na modalidade **B** foram efectuados três tratamentos com um custo total de 186$00. Este valor não foi compensado pelo diferencial das perdas, em relação à testemunha (91$00).

Os custos relativos ás modalidades **D** e **E** foram, respectivamente, de 1440$00 e 720$00. Estes valores também superaram a diferença entre as perdas (Quadro **IV**).

Quanto à modalidade **C**, de referir que dada a pequena diferença das perdas em relação à testemunha, não parece ter sido compensadora a sua utilização. No entanto, não poderá deixar de ser considerada a vantagem do seu reduzido impacto ambiental.

4-Conclusões

Os resultados obtidos no decurso do ensaio sobre a utilização de armadilhas como meio de luta consideram-se preliminares. No entanto, os resultados revelam interesse pela metodologia utilizada, a qual terá alguma viabilidade de aplicação prática em pomares de pequena dimensão. Para uma sua utilização mais generalizada, nomeadamente em pomares do tipo industrial, considera-se ser necessário atender á redução do custo das armadilhas e da mão de obra necessária para a sua aplicação.

A escolha do tipo de armadilhas utilizadas resultou da experiência que tem sido acumulada com os estudos que têm decorrido em citrinos no Algarve sobre a bioecologia de *C. capitata*. No decurso dos ensaios referentes ao presente trabalho, a combinação dos dois tipos de armadilhas utilizadas revelou-se adequada para os objectivos pretendidos.

O tratamento em faixas foi o que revelou maior interesse, pois o seu custo foi pouco superior ao valor das perdas de frutos comercializáveis. De considerar que, na prática, poderá haver vantagens com este tipo de tratamento, se atendermos ao facto de a testemunha se encontrar rodeada por parcelas sujeitas a tratamento consoante as respectivas modalidades. Isto, aliado ao facto da restante plantação ter sido sujeita aos tratamentos realizados pelo agricultor, poderá ter tido influência numa menor incidência dos ataques.

No que respeita ao comportamento, relacionado sobretudo com a actividade de postura das fêmeas, no decurso dos estudos confirmou-se a preferência pela exposição Sul da copa e verificou-se uma maior incidência das picadas na superfície externa inferior dos frutos. Estes dados harmonizam-se com a orientação Sul que deve ser dada à aplicação dos tratamentos em faixas, assim como à colocação de armadilhas para monitorização e estudos de dinâmica de populações.

Perante os resultados obtidos e a experiência adquirida no decurso do estudo, considera-se haver interesse na continuação deste, numa maior escala, ao nível da dimensão da parcela e da diversidade dos tipos de plantações existentes.

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Session 3

Genetic aspects and microorganisms of fruit flies populations

Chairman: CLAUDE LOUIS

Secretaries: A. MALACRIDA
R. WOOD
D. OCHANDO

Report on Session 3

CLAUDE LOUIS; A. MALACRIDA; R. WOOD; D. OCHANDO

Microrganisms

In what concerns microrganism Claude Louis pointed out the important role of fruit flies in transmission of rots caused by fungi and bacteria in respect to the medfly and drosophilas in peach, apple and citrus tree in the mediterranean countries. Attention was also drawn to the acidic rot of grapes transmitted by drosophilas. Further programmes which could be developed include: Field experiments on medfly transmission of microorganisms, including regurgitation beehaviour; comparisons of rots for their attractivity to the medfly, with a view to incorporating them into baits; role of symbiotic microrganisms.

As far as the genetic aspects are concerned, the considered topics were:

1) Analysis of the protein and DNA variability to assess the population genetics of *Ceratitis capitata* (Malacrida A. and Ochando D.)
2) Genetical and cytological studies on the male producting factor (MP) (R.Wood)

Populations genetics

The studies on the medfly population structure have the aim to assess the presence of intraspeciphic differentiation and to infer the route of the invasion of this pest species. Drift and selection are the two evolutionary processes involved in the colonization process. On the basis of the variability data, the medfly seems to have colonized the Mediterranean basin across the Spain.

Male producting factor (MP)

The male producting factor (MP) exerts its effect during sperm production. In the *Ceratitis capitata* strain carrying the MP gene it has been revealed the presence of abnormal spermatozoa and a reduction of spermatozoal number. The excess of males observed in the sex ratio can be explained on the basis that all abnormal and missing spermatozoa are potentially female determining.

A STUDY OF GENE-ENZYME VARIABILITY IN THREE SPANISH POPULATIONS OF *CERATITIS CAPITATA*

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Summary

Genetic variation was examined by using starch-gel electrophoresis at 11 allozyme loci in the Mediterranean fruit fly *Ceratitis capitata*. One laboratory population and two natural populations from the North-east and Mid-east of Spain were studied.

Average heterozygosity was 0.0551. The proportion of polymorphic loci was 56% (99% criterion). The average number of alleles per locus was 1.89.

Concerning the pattern of the variation, the most remarkable finding was the similarity of the configuration of allelic frequencies in our populations.

Our observations support the conclusion that balancing selection is the major factor responsible for the genetic variation observed in *Ceratitis capitata*.

1 - Introduction

Variation is the cornerstone of evolutionary biology. Furthermore, the evolutionary potential of a population can be measured by the amount of genetic variability in that population; the more genetic variation there is, the greater the opportunity for evolution to occur (Ayala, 1965, 1976). It is not surprising, therefore, that variability studies have constituted a very important chapter in evolutionary biology, and specifically in population genetics.

Electrophoresis has become by far the most useful tool for quantifying inherited variation in population genetic studies since its introduction nearly three decades ago (Hubby & Lewontin, 1966; Harris, 1966). Gel electrophoresis techniques provide a simple, unweighted method for quantification of existing variability.

Until recently, however, most applied biologist have not utilized the benefits of evolutionary biology methodologies. In addition, evolutionary biologists have not been interested in species of agricultural importance, probably because it is much easier to cultivate Diptera such as *Drosophila* in the laboratory. Notwithstanding, nowadays, geneticists have become interested in agricultural pests, and applied biologists have realized the relevance of evolutionary biology and its molecular techniques to the problems of the control of pests in agriculture and forestry (Loxdale & Hollander, 1989).

Today, therefore, species of such importance from an agro-economic point of view as is *Ceratitis capitata*, are insufficiently known genetically, and knowledge of the genetic structure of its populations is not widespread. Indeed, in the bibliography only six extensive and systematic works on the populations of *Ceratitis capitata* are available (Huettel *et al.*, 1980; Morgante *et al.*, 1981; Loukas, 1989; Milani *et al.*, 1989; Gasperi *et al.*, 1986, 1991; Malacrida *et al.*, 1992). And of these studies, only one refers to a Spanish population (Loukas, 1989). It is important to note that knowledge about population structure would, in turn, permit indirect knowledge about population dynamic (introgression, genetic drift, colonization or dispersion, gene flow or migration, founder

effect, bottlenecks, etc.). Therefore, information about population structure and dynamic is essential if we want to fight against the pest in a more efficient and environmentally safe manner.

Based on the above considerations, the objetive of the present study has been to investigate, through gel electrophoresis, the genetic variability present in Spanish populations of *Ceratitis capitata*. In addition, the resulting information about the population structure can contribute to clarify whether the migraton pattern of *Ceratitis capitata* from its origin (tropical Africa) to the Northern Mediterranean countries extends throughout the Iberian Peninsula. Last, but not least, with this study we can contribute with new information to the Neutralism-Selectionism controversy through consideration of an organism which has a special ecology and which also has to face specific and extreme environmental conditions such as insecticides.

2 - Materials and Method

Two natural and one laboratory populations were studied, all of them of Spanish origin. These are: the **Gerona** population, from apples of the North-east of Spain (Gerona area); the **Iela** population, from peaches of the Mid-east of Spain (Valencia area); and the **Agronomos** population, developed more than ten years under laboratory conditions. Collections of wild flies from these natural populations were made by harvesting infested fruits (apples and peaches) and allowing the larvae to pupate in the laboratory. The analyzed flies in the **Iela** population were those obtained directly from the fruits. The **Gerona** flies were second generation laboratory flies.

Standard techniques for horizontal starch gel electrophoresis and assay of enzymes were applied to adult flies, in accordance with the procedures described by Ayala *et al.* (1972) with minor changes. One buffer system was always used: gel buffer 76 mM Tris and 5 mM citric acid, pH 8.65; electrode buffer 300 mM boric acid and 60 mM NaOH, pH 8.1 (Poulik, 1957; Ayala *et al.*, 1972).

We studied 11 loci coding for enzymes as follows: esterases, three loci (Est_1, Est_2, Est_3); hexokinases, two loci (Hk_1, Hk_2); and one locus for each of the following enzymes: aldehyde oxidase (*Aox*), fumarase (*Fum*), fructokinase (*Fk*), hydroxybutyrate dehydrogenase (*Hbdh*), isocitrate dehydrogenase (*Idh*) and phosphoglucomutase (*Pgm*). A *Drosophila melanogaster* monomorphic strain ($X9_{SOD}$, provided by F.J. Ayala) was used as control in the gels. This strain has been used as reference "100" for allele designation in all cases.

A sample size of 100 (or as close as possible) was used in the great majority of the cases. Only in the **Gerona** population was it not possible to study two loci (Hk_2 and *Idh*), and for two of the loci, *Aox* and Hk_1, the sample sizes were 37 and 31 respectively.

3 - Results

Table I sumarizes the amount of variation found for 11 loci in each of the three studied populations. For each locus, Table I shows the allelic frequencies, the proportion of the heterozygous individuals observed, the expected according to the assumption of Hardy-Weinberg equilibrium and the number of genomes sampled.

Table I - Allelic frequencies, observed (H_O) and expected (H_E) heterocygotes and sample size (N) at 11 loci in three Spanish populations of *Ceratitis capitata*. ($^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$)

		Populations		
Locus	Allele	Agronomos	Gerona	Iela
Aox	97	0.3600	0	0.1900
	99	0.2600	0	0.5600
	100	0	0	0.0100
	101	0.3800	1.0000	0.2400
	H_O	0.3000***	0	0.3000***
	H_E	0.6584	--	0.5926
	N	100	37	100
Est$_1$	117	1.0000	1.0000	1.0000
	N	100	73	100
Est$_2$	108	0	0.0137	0
	110	1.0000	0.9863	1.0000
	H_O	--	0	--
	N	50	73	73
Est$_3$	98	0.3565	0.1266	0.1450
	100	0.5962	0.8608	0.0400
	102	0.0673	0.0126	0.7950
	104	0	0	0.0200
	H_O	0.2885**	0.2532	0.0900***
	H_E	0.5129	0.2428	0.3450
	N	52	79	100
Fum	103	0	0.0422	0
	104	1.0000	0.9296	1.0000
	105	0	0.0282	0
	H_O	0	0***	0
	H_E	--	0.1333	--
	N	100	71	100
Fk	101	0.0100	0	0
	102	0.9800	1.0000	0.9900
	103	0.0100	0	0.0100
	H_O	0	--	0
	N	100	78	100
Hk$_1$	102	0.9700	1.0000	0.9700
	103	0.0300	0	0.0300
	H_O	0*	0	0*
	H_E	0.0582	--	0.0582
	N	100	31	100

Table I - (cont.)

Locus	Allele	Populations		
		Agronomos	Gerona	Iela
Hk_2	73	0	--	0.0400
	74	0	--	0.0100
	75	1.0000	--	0.9400
	76	0	--	0.0100
	H_O	0	--	0.0800^{-}
	H_E	--	--	0.1146
	N	100	--	100
Hbdh	120	1.0000	1.0000	1.0000
	N	100	81	100
Idh	100	0.7944	--	0.9750
	102	0.0667	--	0.0250
	104	0.1389	--	0
	H_O	0.0667***	--	0.0500
	H_E	0.3452	--	--
	N	90	--	100
Pgm	106	0	0.0305	0
	108	0.1000	0.9695	0.0400
	110	0.9000	0	0.9600
	H_O	0.2000^{-}	0.0610^{-}	0.0600^{-}
	H_E	0.1800	0.0591	0.0768
	N	100	82	100

A survey of Table I shows that there are different kinds of loci, from highly polymorphic to completely monomorphic. We can classify the different loci according to the degree of polymorphism:

1 - clearly polymorphic in all the populations with information: *Est_3*, *Idh* and *Pgm*.

2 - polymorphic loci in one/two population(s) and monomorphic in the other(s): *Aox*, *Fum* and *Hk_2*.

3 - monomorphic loci, either with one fixed allele in the three population: *Est_1* and *Hbdh*, or with the presence of some "rare" alleles in some population(s): *Est_2*, *Fk* and *Hk_1*.

As we can observe, in loci groups 2 and 3, there is always the same allele which has become fixed or which represents a very high frequency in the three populations. However, with respect to the first group of loci, the polymorphic ones, it can be seen in Table I that there exists some heterogenity in the frequency distribution of the alleles; that is, local differences exist.

In Table I the frequency of heterozygotes observed is also given. When data permitted statistical application, we calculated the expected frequencies of heterozygous individuals in Hardy-Weinberg equilibrium. As we can see, in most of the cases,

observed and expected frequencies do not agree. Only with the *Pgm* locus do the three populations show equilibrium frequencies. In the other cases, eight out of ten, are far from Hardy-Weinberg equilibrium. Moreover, the lack of agreement between observed and expected frequencies is due to a defect of heterozygotes.

Table II shows the three usual statistics of genetic variability: H, heterozygosity, the average number of heterozygous individuals; n, average number of alleles per locus; and P, polymorphism, the proportion of polymorphic loci. Two criteria of polymorphism are used here (95% criterion and 99% criterion). The mean heterozygosity for all three populations is 0.0551. The number of alleles per locus is 1.95, and the proportion of polymorphic loci is 29% or 56%, according to these two criteria.

Table II - Parameters of genetic variability in Spanish populations of *Ceratitis capitata*: heterozygosity (H), mean number of alleles per locus and polymorphism at 95% and 99% criteria (P).

	H	n	P	
Populations			95%	99%
Agronomos	0.0777	1.91	0.36	0.55
Gerona	0.0349	1.67	0.25	0.50
Iela	0.0527	2.18	0.27	0.64
$\bar{X}$	0.0551	1.95	0.29	0.56

The **Gerona** population is the less variable of the three populations analyzed with respect to all three statistics. And surprisingly enough, the highest degree of heterozygosity, although not the case for polymorphism or the number of alleles per locus, corresponds to the laboratory population.

The Genetic Distances, D, (Nei, 1972, 1978) between all the pairwise combinations of the three populations, based on the 11 studied loci, are: 0.0533 **Iela-Agronomos**, 0.2731 **Iela-Gerona** and 0.1512 **Gerona-Agronomos**. Clearly, the **Gerona** population is distinct from the other two.

4 - Discussion

The examination of our present results attemps to answer the three questions following:

1 - What are the levels of genetic variability in our populations?
2 - What are the patterns of genetic variability in the studied loci?
3 - What types of processes can be postulated to explain that genetic variability? i.e., what are the selective and/or non-selective forces that can generate the patterns of genetic variability found?

4.1 - Amount of variability.

The results of our survey of allozyme variation in *Ceratitis capitata* in the three populations under analysis are given in Table I. Table II summarizes the values of the three classical statistics used in this kind of studies.

Although only 11 loci have been assayed, these may be considered to be a random sample of the genome. Singh and Rhomberg (1987), who have compiled extensive data about variability and species, concluded that doubling the number of studied loci had no significant effect on the proportion of polymorphic loci or on the mean heterozygosity (although they do make reference to a higher number of loci). Therefore, taking this consideration and the fact that we had a large sample size (about 100 individuals) into account, we think that our data are representative of the real genetic variation in *Ceratitis capitata*.

The main feature of Table I and II is the low genetic variability (few alleles and low heterozygosity). This observation is specially notable because the medfly is an extremely polyphagous species. According to some authors (Powell, 1971; Levinton, 1973; Gillespie & Langley, 1974; Yong, 1992), one would expect a positive correlation between a species' genetic variation and the degree of its environmental diversity. However, comparison of genetic variability levels between the medfly, which has multiple hosts, and some single-host fruit-fly species, shows that the genetic variation of *Ceratitis capitata* populations does not follow these expectations. Data on *Rhagoletis pomonella*, *R. completa* and *Dacus oleae*, which infest walnut and olive trees respectively, give a mean heterozygosity of 0.181, 0.075 and 0.188 (Berlocher & Bush, 1982), higher than the heterozygosity values that we found in our medfly populations (Table II). But our values approach to those found in other fruit flies such as *Anastrepha fraterculus* (Malavasi & Morgante, 1983), were H = 0.050.

Most likely, the ability of *Ceratitis capitata* to feed on many types of fruits requires its adaptation to a variety of these different fruits, thus causing a more restrictive range of variability. This variability is ultimately prevented by the necessary adaption to all and each one of the kinds of fruit, which should imply further selection, i.e., selection over selection.

At any rate, if we compare our populations and the populations of *Ceratitis cappitata* analyzed by other authors, our results are in complete concordance; moreover, the quantity of genetic variability that we detect is somewhat higher than that detected in the other systematic studies concerning this species (Huettel *et al.*, 1980; Morgante *et al.*, 1981; Loukas, 1989; Milani *et al.*, 1989; Malacrida *et al.*, 1992). Huettel *et al.* (1980) found an H value of 0.167 in a South African population, and values between 0.034-0.071 in other populations. The polymorphic loci values were 52% for the S. African population and between 9-17% for the others. The number of alleles per locus varied between 1.1-1.2 in non-African populations and 2.3 for the S. African population. Morgante *et al.* (1981) studied four Brazilian populations and found a heterozygosity value of 0.030. Loukas (1989) found higher values for a South African population (H= 0.234, P= 0.54 and n= 2.7) and lower values ranging from 0.039 to 0.068 of H, 0.20-0.30 of P, and 1.2-1.3 for n, with the exception of a Reunion Island population which showed slightly higher values. Malacrida *et al.* (1992) found values of 0.016-0.114 for H,

0.052-0.36 for P, and n= 1.05-1.44 in Meditarranean populations. For populations of Kenya and Reunion values were higher.

In sum, the values that we found are similar to those detected by other authors for Mediterranean populations; moreover, our data are in the upper end for H, and are clearly higher for P and n.

These results are in agreement with the general rule of a decreasing trend in genetic variability from the source area of a species toward the periphery of the species' range. *Ceratitis capitata* is a colonizing species from a source area located in Central Africa. It has been proposed that the insect first invaded Spain in 1842 and then spread from the Iberian Peninsula into the Northern Mediterranean countries, and on to the Middle East (Hagen *et al.*, 1981). The data from our Spanish populations seem to support this hypothesis; we found a lower degree of variability with respect to the African populations, but a higher degree with respect to the Italian and Greek populations (at least with respect to P and n). Notwithstanding, information obtained from only three populations (only two of which were wild) is not enough to make generalizations and more thorough information about Spanish populations is needed before any well supported deduction could be proposed in relation to the migratory route of the colonizing *Ceratitis capitata*.

Genetic Distances were calculated in order to evaluate the variability differentiation among our populations. The data on other Tephritidae species such as *Rhagoletis* give intraspecific values between 0.001 and 0.025 (Berlocher & Bush, 1982). Information available on *Ceratitis capitata* is limited mainly to the Pavia group (Milani *et al.*, 1989; Gasperi *et al.*, 1991), showing distances ranging between 0.011 and 0.107. In our case, the calculated values are much higher, reaching up to 0.2731 for the Genetic Distance between **Iela** and **Gerona** populations. Values such as that are considered to be subspecies differentiation (Ayala, 1983).

Two main features can be observed in our Genetic Distances: first of all, the laboratory population is more similar to the wild populations than they are to each other; secondly, the **Gerona** population is clearly different from the other two.

The **Gerona** population represents a very different ecological area than the **Iela** population. The latter comes from an area of continental climate, and from peach fruits, while the **Gerona** population comes from a more temperate climate and from apple fruits. This indicates a possible different adaptation to a different ecological niche, which could then imply a variability differentiation between the two populations. The differentiation of the **Gerona** population is due to its lower degree of variability. This low variability can be due to either (1) the study of second generation laboratory flies, as it is well known that immediate adaptation to laboratory conditions causes the death of a large number of flies, or (2) the real lower variability of this population in the wild, because of some processes which eliminate variants. More information is needed about this population in order to come to a further conclusion.

With respect to the laboratory population, the length of time in the laboratory (more than 10 years), and the provision of "fresh" flies three years before scanning, may have helped in the positive adaptation to laboratory conditions. This positive adaptation would allow the population to stand a certain degree of variability, without excluding the

possibility of the action of some kind of balancing selection. Tsakas and Zouros (1980) found that laboratory populations of *Dacus oleae* show higher levels of genetic variation than do wild populations, specifically in the increase of heterozygosity.

4.2 - Patterns of variability.

On the other hand, that some gene loci are more variable than others was to be expected. What is remarkable is that all loci are strikingly similar in the pattern of variation (Table I). Basically, three generalizations can be made:

1 - At any given locus, one and the same particullar allele is the most common, or even the only one, in the three studied populations (with three exceptions: *Aox*, Est_3 and *Pgm*).

2 - The gene frequencies are not uniform in the three populations; there is some "geographic" differentiation (we can consider the laboratory population to be another "geographic area").

3 - The heterozygosity frequencies, were data permitted analysis, are not in agreement with the expected equilibrium of Hardy-Weinberg heterozygosities, with some exceptions (Est_3 in **Gerona**, Hk_2 in **Iela**, and *Pgm* in all three populations).

It has been sugessted that most of the genetic variability existing in natural populations, particularly that variability observed at the molecular level, may be adaptively neutral (King & Jukes, 1969; Kimura, 1983; Nei & Koehn, 1983). The frequencies of adaptively neutral alleles are governed not by natural selection but by the random process of sampling errors which occur every generation in any finite population. If, as postulated by the theory of Neutralism, allelic frequencies are the result of sampling errors, these allelic frequencies should be completely uncorrelated among populations. That is, different neutral alleles should occur in different populations or they should occur in frequencies completely uncorrelated. This expectation is in clear contrast with our results. As shown in Table I, at every locus studied there is a remarkable similarity in the configuration of allelic frequencies in the three studied populations. In two completely monomorphic and five partially polymorphic loci, the same allele has been fixed or shows a fairly higher frequency over the others in all three analyzed populations.

But if a certain amount of migration occurs between neighboring populations, local populations would represent samples of a single population. Similar allelic frequencies would then occur in all populations interconnected by migration. However, this explanation encounters serious difficulties in our case for several reasons: (1) we have analyzed a laboratory population that obviously cannot receive any migration from outside, and, nonetheless, this population shows a similar pattern of variation to the other two populations; (2) in spite of the main similarity, some "geographic" differentiation exists in several loci.

In other words, as gene flow and genetic drift are expected to affect all loci similarly, the presence of non-homogenity in the data would indicate the operation of natural selection. Furthermore, as we find (where the data made possible its statistical utilization) that heterozygosities are not in agreement with Hardy-Weinberg equilibrium expectation, there must be some process acting on our variability. Our populations are of a large size, as demonstrated by the distribution of allelic frequencies. This is U-

shaped, which is expected in large size populations (Nei *et al.*, 1975; Huettel *et al.*, 1980). Thus, genetic drift for small size effect is not possible. This means that the process acting on our populations must be selection. Moreover, in spite of the proposal of some authors as to the generalization of heterotic balancing selection, in our case, in agreement with Singh and Rhomberg (1987), there may be diversifying selection in which selection coefficients vary "geographically", the process acting. In sum, diversifying selection must be the most important form of balancing selection in the studied populations.

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POPULATION GENETIC AND GENOME ANALYSES ON *CERATITIS CAPITATA*

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Biochemical and molecular markers (RAPD) were used for population genetic and genome analyses on *Ceratitis capitata*. At the population level, biochemical markers permit recognition of the presence of geographical genetic heterogeneity, and provide information on the relative contribution of different types of evolutionary force during the process of colonization. Polymorphisms in the genomic fingerprints, generated by the RAPD approach, provide a tool for improving the significance of estimates of genetic relatedness between Medfly populations and for distinguishing between slightly divergent flies.

For genome organization analysis, biochemical markers provide information on the relationship between the genome structure and the genome function. The RAPD approach provide a new tool for exploring and completely mapping the Medfly genome.

POTENTIAL FOR DISTORTING SEX RATIO BY MEIOTIC DRIVE GENES

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Summary

We have investigated the action of meiotic drive genes that distort sex ratio in favour of males in the mosquito *Aedes aegypti* (Linn.) and the Mediterranean fruit fly (medfly) *Ceratitis capitata* (Wied.). The genes D (*Ae. aegypti*) and MP (*C. capitata*) are each inherited only through the male line, exerting their effect by reducing the production of functional X-bearing spermatozoa. The action of D is associated with premature senescence of the testes, leading to a reduction in sperm density and the appearance of structural abnormalities such as double tail elements with extra mitochondrial derivatives and axonemes, revealed by electron microscopy. Studies on the action of MP show a similar picture of sperm loss and abnormality in putative X-bearing spermatozoa. Interesting extra information is provided with respect to MP, connected with the observation of discrete 'clones' (cysts) of spermatozoa (maximum number = 256), each enclosed in a cyst wall and derived from a single primordial germ cell. The proportion of missing/abnormal spermatozoa in cysts of MP testes varies widely, from zero (256 spermatozoa) to more than 50% (<128 spermatozoa), even within a single testis. The cause of this variation has yet to be disclosed but the answer, once known, may open the way to increasing the expression of the MP gene. Similar cysts in *Ae. aegypti*, with up to 512 spermatozoa, are less stable and therefore not easy to investigate. Proposals are made for using techniques of molecular genetics to investigate the action of D and MP, with a view to maximising their expression and thereby increasing the potential of these two genes as pest control agents or genetic sexing mechanisms.

1 Introduction

Plans to develop genetic methods of pest and vector control have sometimes met with disappointment (Curtis 1985). The wild insect has proven more resistant to genetic manipulations than was foreseen. Problems and setbacks have been experienced even with the sterile insect technique (SIT) although triumphantly overcome in the case of the screw worm fly *Cochliomyia hominivorax*, the Mediterranean fruit fly (medfly) *Ceratitis capitata* and several other species of fruit flies. Genetics is also being applied, with great benefit, in the development of genetic sexing techniques for mass producing the very large numbers of males required for SIT (IAEA 1990). Plans to advance in this and other areas of genetic manipulation must now take account of the rapid advances being made in molecular genetics (Eggleston 1991). One area for future exploitation is the phenomenon of meiotic drive, the subject of this paper.

Meiotic drive was defined by Sandler and Novitski (1957) as a distortion of the 1:1 segregation predicted by Mendel's first law, resulting in excess of gametes of the 'driven' genotype (Lyttle 1991). Meiotic drive genes may be autosomal or sex linked. A Y-linked gene D, which increases the proportion of male-determining gametes is known in the mosquito *Aedes aegypti* (Wood and Newton 1991) and a similar gene, MP, has been identified in *C. capitata* (Wood et al 1987, Wood and Shahjahan 1988). D and MP each show a pattern of inheritance characteristic of genes closely linked with the male determining locus, being transmitted holandrically ie only through the male line.

D is present in a suppressed state in many wild *Ae. aegypti* populations, commonly in West Africa and Central America. It is often expressed in the F_2

from outcrosses of wild males to females from strains of a different geographical origin. It acts only in coupling with the male determining locus M (genotype MD), causing X chromosomes, with which MD Y chromosomes are paired at male meiosis, to fragment into two or more pieces. The result is a reduction in female-determining spermatozoa (Newton et al 1976). The genetic control of the system, which includes suppressors, involves at least two loci apart from M and D (Wood and Ouda 1987, Wood and Newton 1991).

The MP factor in *C. capitata* was discovered by the appearance of a male-distorted sex ratio in progeny derived from a single X-ray exposed male. The phenotype has since been selected to produce lines yielding 30-35% female (Rendon Arana 1991). A consistently distorted sex ratio can also be observed in some inbred *Ae aegypti* strains carrying the D gene (Wood and Ouda 1987, Owusu-Daaku et al 1993). In both species, such strains may eventually, after 10 or more generations, revert towards a normal sex ratio, as modifier genes (suppressors) are selected.

The reduction in sperm density which results from meiotic drive by the MD Y chromosome was studied by George (1981). Using electron microscopy he showed that not only were sperms lost but a proportion of those remaining were structurally abnormal. In this paper, we report an extension of this line of work, studies of spermiogenesis on both D males and MP males, to provide quantitative data on sperm density and abnormality. The aim has been to provide fundamental information on which to base future attempts to manipulate the D and MP genes for controlling these two dangerous insects.

2 Materials and Methods

Strains of Aedes aegypti: Crosses were made between females of an Australian strain (THURSDAY ISLAND) and males of a strain of Trinidad origin (T8). THURSDAY ISLAND females were from lines selected for high sensitivity to D; T8 males carried D on all Y chromosomes. Progenies from single pair crosses Th.I.oxT8o were maintained as separate lines for 10 generations. Most tests reported here were carried out on line 68, producing 29±10%o. A highly unbred strain 3M, showing a normal sex ratio and no evidence of D, was used as the control (Owusu-Daaku et al 1993).

Strains of Ceratitis capitata: An inbred MP line (H1-18-27/9) produced 35±5%o at the time of study. Two strains with normal sex ratios were used as controls: SEIBERSDORF and DOUBLE CHAETA

The rearing procedures and EM techniques for each species are described by Owusu-Daaku et al (1993) and Rendón Arana (1991).

3 Results

The tail of a normal spermatozoan of *Ae aegypti* shows a single axial filament and two accompanying structures, one on either side, called mitochondrial derivatives. Normal spermatozoa keep this form until the natural process of senescence begins, at about 7 days after males emerge from the pupa. The first signs of senescence are observed by abnormalities among spermatozoa at the distal (posterior) end of the testis. The process is characterised by a disintegration of the wall of the spermatozoon, the release of its contents, accumulation of the scattered contents of many spermatozoa into 'waste bags' and sometimes by the formation of compound spermatozoa with two or more axonemes and three or more mitochrondial derivatives, possibly caused by the fusion of two or more degenerating spermatozoa.

In D males, senescence occurs prematurely. Signs of it are observed within the first four days after emergence, and even as early as emergence itself in lines selected for high X sensitivity to D. It may be assumed that most of the spermatozoa which are prematurely destroyed or abnormal are X-bearing, ie female determining. A study by Newton et al (1976) showed that

the reduction in sperm density is insufficient to provide the complete explanation for the depletion of females observed in the progeny of such males (Table 1). EM studies have revealed a significant proportion of the remaining spermatozoa to be grossly abnormal (George 1981, Owusu-Daaku, unpublished) although this proportion has yet to be defined precisely (Table 1).

The premature loss or abnormality of X-bearing spermatozoa is explained by events occurring earlier, during spermatogenesis. Newton et al (1976) observed deletions in a large proportion of X chromosomes caused by fragmentation during the first division of meiosis. The extent of observed fragmentation was more than enough to explain the deficiency of spermatozoa and almost, although not quite, sufficient to explain the loss of females (Table 1). It may be assumed that the cytological examination missed a small minority of the breaks that had taken place.

In the young testes of *Ae aegypti*, spermatozoa are seen to be arranged in cysts, those in the same cyst assumed to be the products of a single primordial germ cell. The number per cyst in this species appears to assume a maximum of 512, ie the result of 6 mitotic divisions plus the two divisions of meiosis. The intact cysts are rarely observed in section, however, because the cyst wall become broken down, resulting in the merging of spermatozoa from different cysts.

C. capitata cysts are more stable, which is a great advantage when studying the impact of meiotic drive from the MP factor on sperm depletion or structural abnormality. The normal number of spermatozoa per cyst in C. capitata is 256, the result of five mitoses plus meiosis. In MP males the number of spermatozoa is reduced and some of those remaining are abnormal, showing the same structural changes observed in *Ae aegypti*. The advantage in *C. capitata* is that the number per cyst can be counted. By the number of normal spermatozoa per cyst in MP males, it can readily be shown that the average figure corresponds almost precisely with the loss to be expected from the sex ratio distortion observed in the progeny of such males (Table 1), assuming that, apart from a small control mortality, it is the X-bearing spermatozoa which are lost or abnormal.

[Slides of EM micrographs of spermiogenesis in both species will be shown]

One observation to be noted is the wide variation observed between cysts within the same MP testis. Some show 50% spermatozoa missing or abnormal (100% expression of MP); others show the full complement of apparently normal spermatozoa (zero expression of MP). Because the spermatozoa from different cysts become mixed together, it is the average content of spermatozoa per cyst which is reflected in the sex ratio (Table 1).

4 Conclusions

Within the sphere of pest or vector control, sex linked meiotic drive genes have the potential to be exploited in one of three ways: to impose a genetic load on a pest/vector population to a degree from which it cannot recover; to act as a transporting mechanisms for carrying a linked, 'useful' gene into a population to make it harmless or more easily controlled; to act as a genetic sexing technique for mass producing males for SIT. Studies with caged populations have shown that the D gene in *Ae aegypti* has potential in each of these three respects (Curtis et al 1976, Wood et al 1977). To be of really practical use, however, a sex linked meiotic drive system, such as D or MP, needs to be conditional in its action, in a way that can easily be controlled. The need to breed such a strain in large numbers demands culture conditions in which the gene is not expressed. Then, with an appropriate stimulus applied, the strain can be transformed, as required, to produce only males.

No conditioning stimulus is know in *Ae aegypti* but studies on *C capitata* have shown that certain temperature treatments during pupal development can modify the sex ratio distortion caused by MP (Wood et al 1987, Wood and Shahjahan 1988). The critical period for temperature sensitivity has been identified as 72-96 h into pupal development at 25°C, the time when spermatogenesis begins in the male pupa. The effect observed needs to be greatly enhanced, however, before it can be of practical value.

We have noted that MP is expressed to its maximum (50% loss of spermatozoa) in some testicular cysts while, in the same testis, other cysts show no expression at all (zero loss). One approach to making MP conditional in its action may be to understand and exploit the cause of the variation between cysts of the same genotype, assuming that there is a definable 'cause'. It may be discovered that the variation is stochastic, ie due to 'local accidents of development' (Falconer 1960 p14), but the possibility of some definable stimulus, which switches on the MP genes in some cysts but not others, is intriguing and seems worth investigation. Evidence so far indicates that the variation is not defined by the age of the cyst. When spermatozoa of the first formed cysts were sampled by mating newly emerged males, the sex ratio in the progeny corresponded with the sex ratio predicted from the average degree of sperm loss/abnormality in the cysts examined from the testes of unmated sibs (Table 1). Variation between cysts is a subject for our future attention, in conjunction with further studies on temperature sensitivity at meiosis.

Progress in enhancing the potential of meiotic drive genes must surely benefit from future developments at the molecular level. With respect to D, we already have information about its map position in relation to M and to a closely linked band of guanine-cytosine enriched DNA, identified by Hoechst-33258 fluorescence (Wallace and Newton 1987, Wood and Newton 1991). With the D gene cloned, which must be the primary aim, it will be possible to examine its structure and, perhaps, thereby gain some idea of its action.

Many questions about D remain unanswered: what causes X chromosomes to fragment at four discrete site? Does the D product 'attack' DNA at these four points? Does it attack some other constituent of the chromosome? Or is D a transposable element which inserts at these four positions? And what protects the Y chromosome from the action of D? As with D, so with MP, questions arise which can only be answered by molecular study.

No doubt much of value can be gained by taking advantage of the rapid progress being made in the study of meiotic drive in *Drosophila*. The D gene of the *Drosophila* SD system has recently been cloned and sequenced (Wu 1993). It may prove an effective probe for locating D in *Ae aegypti* or MP in *C. capitata*. Also investigated in *Drosophila* has been the locus R which control response to D. Wu and his colleagues have shown R to be a satellite DNA array with sensitivity related to copy number (Wu 1993). R seems to be homologous with m in *Ae aegypti*. 'Alleles' (different copy numbers ?) of m vary in sensitivity to MD (Wood and Newton 1991).

Clearly the potential of genetic manipulation of such phenomena as meiotic drive, as an approach to pest control, has hardly yet been exploited. While we may be sure that there is no 'quick fit' solution to genetic control, we are bound to explore its possibilities with all the resources open to us. Only by being adventurous in one science, shall we keep one step ahead of the insects.

Acknowledgements

Thanks are expressed to Dr E Boller (Wädenswil), Dr Y Rössler (Tel Aviv) and Dr L Munstermann (Notre Dame, Indiana) for supplying us with the SEIBERSDORF and DOUBLE CHAETA strains of C capitata and the 3M strain of Ae aegypti, and to Miss J Hallows for technical assistance.

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Table 1

Percentage of females observed in the progeny of Aedes aegypti males carrying the Y-linked D gene and Ceratitis capitata males carrying the Y-linked MP gene, compared with percentage of females expected on the basis of (1) observed fragmentation of X and Y chromosomes at spermatogenesis (2) sperm depletion (density compared with males not carrying these genes) (3) sperm depletion + sperm abnormality.

Species	Meiotic drive gene	Percentage female pupae or newly emerged adults				Reference
		Predicted by X or Y breakage at meiosis 1	Predicted by sperm density (including abnormal sperm)	Predicted by density of normal sperm	Actually observed	
Aedes aegypti	D	8.6	21	<21	3.8	Newton et al 1976
Ceratitis capitata	MP	-	42.9	37.7	36.7	Rendon Arana 1991

LA RÉGURGITATION CHEZ *CERATITIS CAPITATA* WIED. TRANSMISSION DE "POURRITURES DES FRUITS"

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REGURGITATION BEHAVIOUR AND THE TRANSMISSION OF FRUIT ROT BY *CERATITIS CAPITATA* WIED.

Summary

The Medfly, *Ceratitis capitata* Wiedemann, is generally studied within the context of a primary pest. Here we describe its role as a vector of fruit rot.

The feeding behaviour of *C. capitata* under laboratory conditions follows the general activity pattern, with maximum activity occurring in the first six hours of the photophase. The average quantity of liquid ingested by an insect is 2.5µl.

The results obtained, using *Rhizopus stolonifer* as a model, proved that *C. capitata* may act as a vector of fruit rot either through the passive transport of spores on the body, or by feeding with regurgitation or defecation.

The importance of regurgitation in the feeding behaviour, and thus the transmission of fruit rot, together with the increase in population numbers of this insect, suggest that we must reevaluate the economic importance of this pest.

Résumé

La Mouche Méditerranéenne des Fruits, *Ceratitis capitata* Wiedemann, est le plus souvent considérée pour son rôle de "ravageur primaire". Nous présentons ici son rôle de vecteur de maladies des fruits.

L'étude éthologique descriptive du phénomène de régurgitation chez *C. capitata* menée en conditions de laboratoire montre que ce comportement suit un rythme proche du rythme d'activité général de l'insecte, la période de "régurgitation maximale" se produisant durant les six premières heures d'éclairement. L'analyse des quantités de solution nutritive ingérées par individu fait apparaître une moyenne de 2,5µl.

Les résultats obtenus, en utilisant *Rhizopus stolonifer* comme modèle, montrent que *C. capitata* peut être à l'origine de la transmission d'agents pathogènes de végétaux cultivés.

Cette transmission peut être externe (par transport sur le corps de l'insecte), ou interne (notamment par régurgitation ou par passage dans le tube digestif et rejet par les feces).

L'étude éthologique du phénomène de régurgitation chez *C. capitata* ainsi que la recrudescence des populations dans le sud de la France doivent nous amener à revoir à la hausse la nature des dégâts pouvant être occasionnés par cette "mouche des fruits d'importance économique".

1 - Introduction

La Mouche Méditerranéenne des Fruits est un des ravageurs les plus importants dans le Monde.

Liquido et al. (1990) considèrent que c'est probablement le plus polyphage des *Trypetidae* (ou *Tephritidae*).

Ce ravageur est le plus souvent étudié pour les dégâts qu'il occasionne dans les vergers.

Les résultats présentés ici s'intègrent dans un programme de recherche faisant l'objet d'une thèse de doctorat sur le comportement de régurgitation de *C. capitata*.

L'agent pathogène est *Rhizopus stolonifer*, responsable de la pourriture molle des fruits après récolte. Ce champignon a été choisi pour sa rapidité de développement, et ses spores aisément reconnaissables en microscopie électronique à balayage. La souche nous a été fournie par la Station de Technologie des Produits Végétaux de l'INRA Montfavet (France).

La cage de test est réalisée en Altuglass (5mm), divisée en deux parties cubiques (L=100mm) jointes par un tube d'Altuglass (L=150mm ; Ø=37mm). Le dispositif est stérilisé avant chaque utilisation.

Le protocole est celui utilisé par Louis et al. (1989) sur *Drosophila melanogaster*. Un milieu de culture gélosé stérile est placé dans le premier compartiment du dispositif et un milieu sporulé dans le second. Les deux milieux sont ouverts. Dix individus (5 mâles et 5 femelles) sont placés dans la partie "contaminée". Une heure après, les insectes sont récupérés, disséqués, la tête et les pattes étant conservés à -80°C en vue d'être analysés en microscopie électronique à balayage. Les milieux de culture sont fermés et placés à 28°C durant 48h pour incubation. La même manipulation est exécutée sans insectes et utilisée comme "témoin". Cette expérimentation a fait l'objet de dix répétitions.

Les parties disséquées (tête et pattes des insectes) sont placées sur des porte-échantillons puis métallisés à l'or fin par "sputtering". Cinq porte-échantillons (soit dix insectes) sont ainsi préparés. Dix insectes issus de l'élevage sont utilisés comme "lot témoin". Les porte-échantillons ainsi préparés sont ensuite examinés en microscopie électronique à balayage sur un appareil de type Philips XL30.

2 - 4 Vection par transmission "interne"

Les insectes utilisés sont issus de l'élevage de masse et soumis à un jeûne de 24 heures afin d'augmenter leur attirance vis à vis de la solution nutritive.

Le liquide nutritif est constitué d'une solution de sucrose à 10% contenant des spores de *Rhizopus stolonifer* en suspension.

Cent insectes sont placés individuellement dans des boîtes de Pétri (Ø=100mm ; h=10mm). La solution nutritive "sporulée" est fournie aux insectes par un tube micro-capillaire (5µl) afin de permettre la prise de nourriture, mais d'exclure toute possibilité de contamination externe du reste du corps. Une fois l'insecte nourri, le micro-capillaire est enlevé. Le processus est surveillé en permanence durant une heure, nous permettant ainsi de distinguer les régurgitats des feces déposés à la surface de la boîte de Pétri. Les régurgitats et les feces sont récoltés séparément au moyen d'une pipette Pasteur étirée à la flamme. Les régurgitats sont collectés durant les dix premières minutes, les feces après une demie-heure ou plus après la prise de nourriture. Des milieux de culture stériles sont inoculés avec les régurgitats d'une part et les feces d'autre part et sont placés 48h à 28°C pour incubation. Le même protocole est exécuté en utilisant une solution nutritive à 10% de sucrose ne contenant pas de spores, comme "témoin".

3 - Résultats

3 - 1 Rythmes de régurgitation et réabsorption

Le dénombrement, durant les douze heures d'éclairement, du nombre de régurgitats déposés à la surface du substrat permet d'obtenir un rythme de régurgitation proche du rythme d'activité général de l'insecte décrit par Dridi en 1990. Ce rythme présente l'allure d'une Courbe de Gauss bimodale s'étalant sur toute la durée d'éclairement (Fig. 1). Cette courbe présente son maximum vers la cinquième heure d'éclairement puis décroît pour présenter un deuxième pic de moindre importance vers la dixième heure.

Il est intéressant de noter que tous les essais nocturnes réalisés ont montré une activité nulle au niveau de la régurgitation, donc de la prise de nourriture, confirmant ainsi les moeurs exclusivement diurnes de cet insecte.

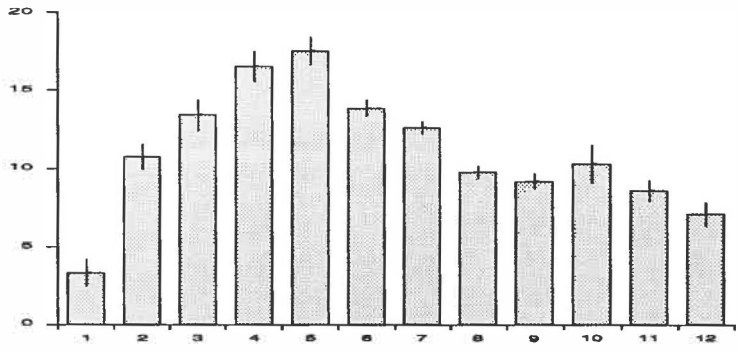

Fig. 1 - Rythme moyen de régurgitation estimé en nombre de gouttes régurgitées, par individu et par heure.
Abscisse : heures d'éclairement - Ordonnée : nombre moyen de gouttes régurgitées (moyenne établie sur 2000 insectes) - Barre : erreur standard

Le rythme de réabsorption semble "calqué" sur celui de régurgitation (Fig. 2). Il apparaît donc que seule une infime quantité des régurgitats déposés sur le substrat n'est pas réabsorbée donc réutilisée. Il nous a été possible de constater que les régurgitats d'un individu peuvent être réabsorbés par un autre individu, induisant donc la possibilité ponctuelle de trophallaxie.

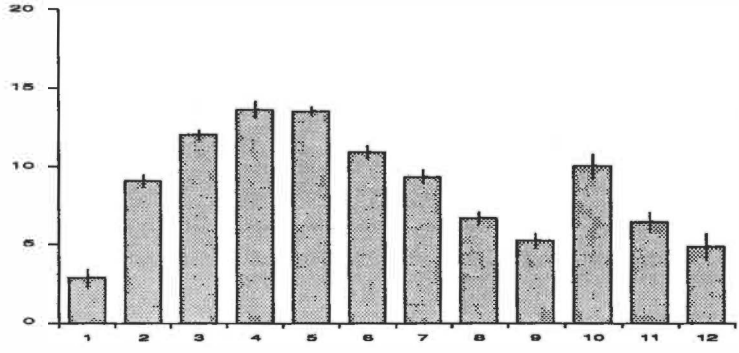

Fig. 2 - Rythme moyen de réabsorption estimé en nombre de gouttes réabsorbées, par individu et par heure.
Abscisse : heures d'éclairement - Ordonnée : nombre moyen de gouttes réabsorbées (moyenne établie sur 2000 insectes) - Barre : erreur standard

3 - 2 Estimation de la quantité de nourriture liquide ingérée

Ce test consistait à estimer la quantité de nourriture liquide pouvant être ingérée en une seule prise par un individu, en vue de connaître les possibilités d'absorption en grande quantité d'un agent pathogène se trouvant sur la plaie d'un fruit blessé.

Aucune différence significative n'étant apparue entre les quantités absorbées par les individus mâles et femelles, les résultats présentés ici portent sur l'ensemble de la population.

Les histogrammes obtenus à trois températures différentes (21, 26 et 28°C) sur des insectes d'élevage (Fig. 3) font apparaître un mode à 2,5µl (de 36 à 40% des insectes testés) autour duquel se répartissent, plus ou moins uniformément selon les températures, les autres individus.

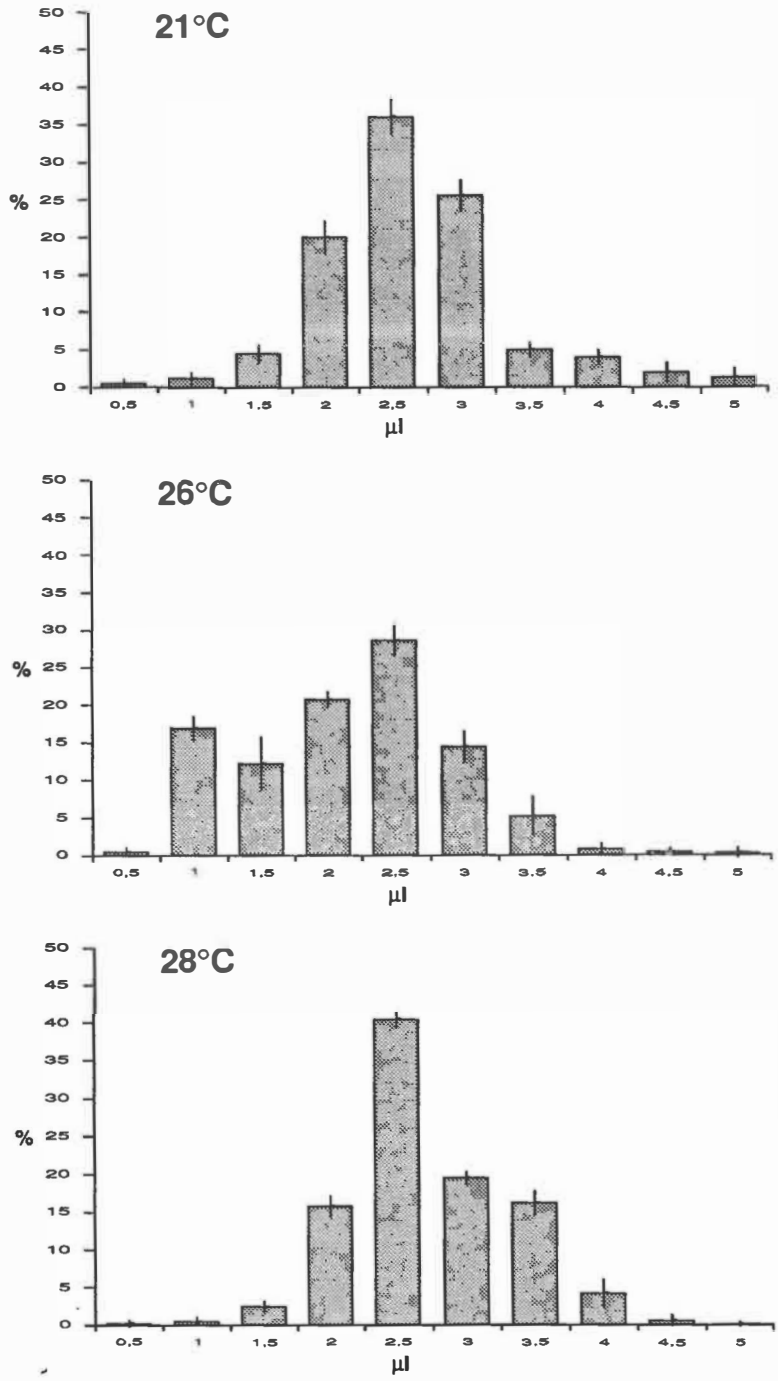

Fig. 3 - Estimation de la quantité de nourriture liquide absorbée en une seule prise par un individu après 24 heures de jeûne, à trois températures différentes.

Abscisse : quantité absorbée en µl - Ordonnée : pourcentage moyen d'individus (moyenne établie sur 1000 insectes) - Barre : erreur standard

Les individus absorbant 5µl de la solution nutritive périssent rapidement par déchirement de l'abdomen.

La quantité moyenne absorbée autorise donc l'absorption en solution d'une grande quantité de spores de champignon ou autre agent pathogène.

3 - 3 Vection par transmission "externe"

Après incubation, tous les milieux initialement stériles présentent un développement de *Rhizopus stolonifer* (caractérisé par des stolons blancs à la surface du milieu), et ceci uniquement dans les dispositifs contenant des insectes, et non dans le témoin, et ce lors des dix répétitions.

Ces résultats excluent la transmission aérienne ou due à des facteurs non relatifs aux insectes (dans nos conditions expérimentales), et confirment l'aptitude de la Mouche méditerranéenne des fruits à agir en tant que vecteur mécanique (ou "externe").

L'analyse en microscopie électronique à balayage révèle la présence de spores de *Rhizopus stolonifer* sur les palpes labiaux, le proboscis et les pattes des insectes contaminés (Fig. 4). Aucune spore n'a pu être mise en évidence sur les insectes d'élevage utilisés comme témoin.

Sur les tarses, les spores apparaissent comme "piégées" entre les poils. La présence de spores sur les palpes labiaux prouve que le milieu sporulé a bien été prospecté par les insectes, nous amenant à penser que la présence de ce champignon pourrait, in Natura, augmenter l'attractivité des fruits pour C. *capitata*, préférant ainsi des fruits infectés à des fruits sains, comme cela a été montré par Louis et al. (1989) pour le genre *Drosophila*.

3 - 4 Vection par transmission "interne"

Tous les milieux inoculés avec les régurgitats obtenus par des mouches nourries avec la solution sporulée montrent un développement de *Rhizopus stolonifer* après 48h à 28°C, mais aucun développement avec le témoin.

Les mêmes résultats sont obtenus avec tous les milieux de culture ensemencés avec les feces.

4 - Conclusions

La régurgitation chez les *Trypetidae* est un comportement qui n'avait été, jusqu'à lors, que très peu étudié, tout au plus mentionnait-on son existence comme un obstacle à surmonter, voire même insurmontable dans le cadre de certains protocoles (Cordes, 1969).

En 1947, Hanna, parlant de C. *capitata* affirmait ceci : "La mouche est capable de liquéfier des substances solides sèches comme le sucre, par régurgitation de liquide à partir de l'intestin". Ce phénomène fut mis en évidence en nourrissant quelques mouches d'une solution colorée puis en mettant à leur disposition un buvard imbibé de solution sucrée, puis désséché. En récupérant cette nourriture, les mouches régurgitèrent et déposèrent sur le papier des gouttes colorées.

Cordes, en 1969, lors d'une expérimentation sur le marquage radioactif de C. *capitata* par une solution de ^{32}P, vit rapidement tous les individus marqués. Ses conclusions furent alors qu'un marquage "propre" par une solution radioactive était rendu impossible par une bioélimination trop rapide du radioélément, notamment par la régurgitation.

A l'opposé, certains travaux comme ceux de Nestel et al. (1985), sont rendus inutilisables par l'ignorance de ce comportement. Les auteurs, étudiant la régulation du sucrose ingéré en solution par C. *capitata*, prirent en compte un nombre impressionnant de paramètres tels que l'évaporation de la solution dans le nourrissoir ou l'humidité relative, mais ne tinrent aucun compte de la régurgitation : ils travaillaient sur des cages grillagées, rendant impossible la détection des régurgitats translucides.

Il faut attendre les travaux de Hendrichs (1986) et Drew et Lloyd (1987) ou de Hendrichs et al. (1992, 1993) pour que ce phénomène fasse l'objet d'études plus approfondies.

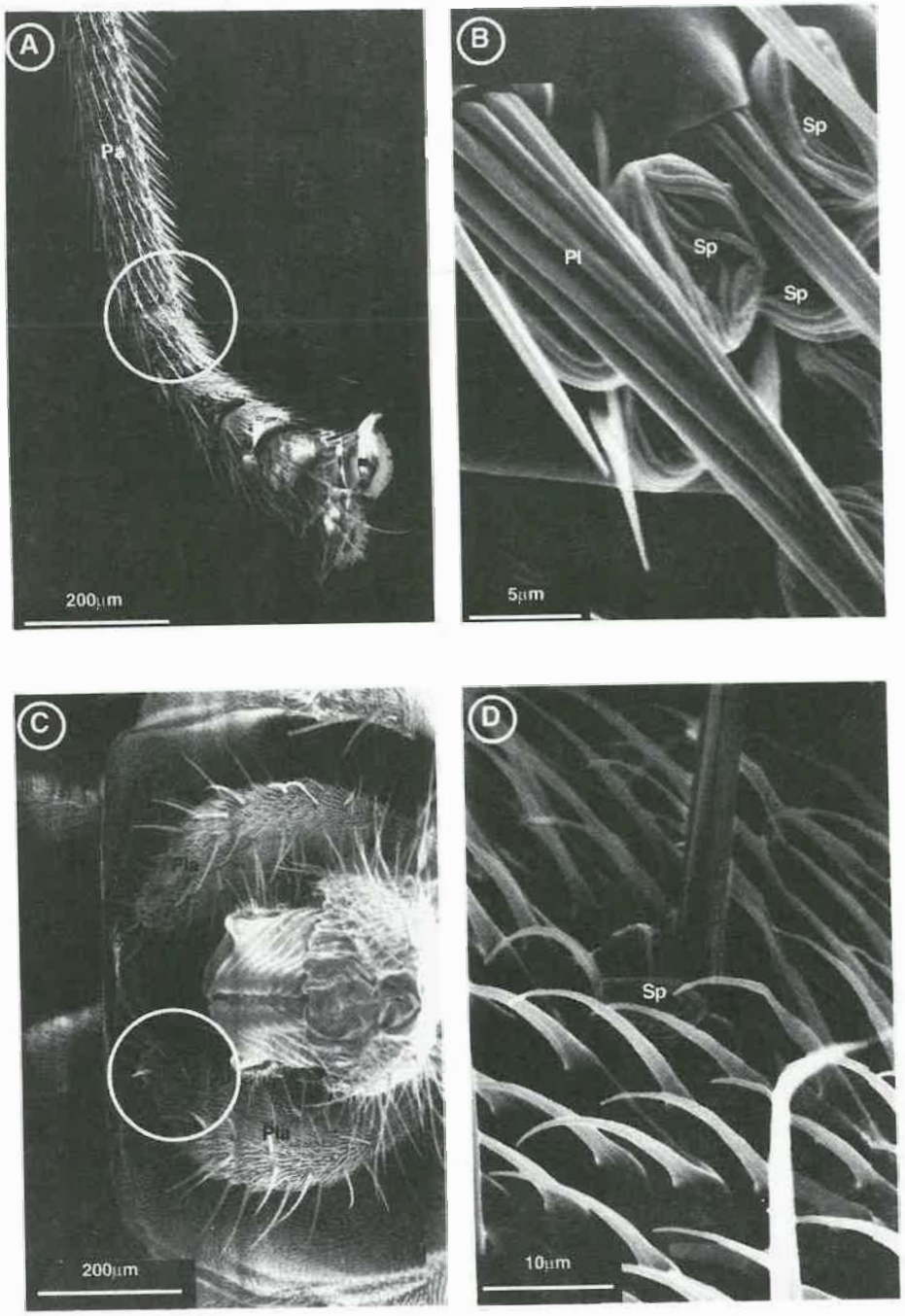

Fig. 4 - Mise en évidence en microscopie électronique à balayage des spores de *Rhizopus stolonifer* sur les pattes (A et B) et sur les palpes labiaux (C et D) de *Ceratitis capitata*.
Pa : patte - Pla : palpe labial - Pl : poil - Sp : spore - Barre : échelle

La régurgitation est un phénomène relativement répandu dans le monde des Insectes, et plus particulièrement chez les Diptères.

Séguy (1967) dans son "Dictionnaire des termes d'entomologie" définit la régurgitation ainsi : "échange de nourriture d'un insecte à un autre de la même espèce ou d'une espèce différente". Cette définition introduit la notion de trophallaxie qui n'est, à notre avis, pas toujours évidente.

Hendrichs et al. (1992) apportent une définition plus précisément adaptée aux *Trypetidae*. Selon cet auteur, le phénomène de régurgitation comprend trois comportements distincts : expulsion orale du contenu liquide du jabot à la surface des pièces buccales, exposant ainsi la gouttelette, située à l'extrémité du proboscis étiré, à l'air, donc à l'évaporation ; détente et rétraction rythmique du proboscis, phénomène durant lequel le liquide apparaît "tournoyant" au sein de la goutte ainsi formée, c'est le phénomène de "pumping" ; et éventuellement, ré-ingestion du liquide. A ces trois phases, nous ajouterons le dépôt de gouttelettes sous la forme de chapelets à la surface du substrat.

Nous présentons ici dans un premier temps une étude "descriptive" succincte de ce phénomène et, dans un second temps, une étude "appliquée" mettant en évidence les potentialités de vection d'agents pathogènes des végétaux par *C. capitata*.

2 - Matériels et méthodes

2 - 1 Rythmes de régurgitation et réabsorption

Les insectes utilisés sont issus de l'élevage mené à la Station de Recherches de Zoologie et d'Apidologie de l'INRA Montfavet (France), sous conditions constantes (T=28°C ; L/D : 12/12).

Dix couples d'insectes sont placés dans une cage en Altuglass (L=100mm). En son centre est placé un coton dentaire imbibé de nourriture liquide à base de miel et d'autolysat de protéine à laquelle a été rajoutée de la Rhodamine (marqueur fluorescent).

Le dispositif est ainsi laissé durant une heure. Les insectes sont replacés dans une cage neuve toutes les heures durant les douze heures d'éclairement.

Chaque cage est analysée sur une plaque à ultra violets, la Rhodamine permettant de distinguer les gouttelettes régurgitées, et l'empreinte des gouttelettes réabsorbées. Le nombre de gouttes de chaque type est comptabilisé.

Ce test a fait l'objet de dix répétitions pour chaque heure d'éclairement.

Cette méthode d'approche indirecte des quantités régurgitées, bien que relativement imprécise, a été choisie car, sur le plan technique, aucune autre méthode ne permettait alors une récupération totale des régurgitats.

2 - 2 Estimation de la quantité de nourriture liquide ingérée

Les insectes utilisés sont issus de l'élevage de masse et subissent un jeûne de 24 heures à la température à laquelle se déroulera le test (21, 26 ou 28°C).

Les insectes sont placés individuellement dans des cages cylindriques réalisées en Altuglass. La nourriture liquide leur est dispensée par l'intermédiaire d'un microcapillaire de 5µl.

Dès que l'insecte quitte le microcapillaire, ce dernier est ôté de la cage, interdisant ainsi une deuxième prise de nourriture. Il est alors comparé à un tube étalonné, permettant d'estimer, avec une certaine précision, la quantité de liquide ingérée en une seule prise de nourriture.

2 - 3 Vection par transmission "externe"

Les insectes utilisés sont issus de larves obtenues à partir de fruits infestés (*Malus sylvestris* var Grany-Smith) récoltés dans le sud de la France durant l'automne 91 (Cayol et Causse, 1993).

Les différents mécanismes de transmission, quant à eux, ont déjà été étudiés pour quelques espèces de *Diptera*.

Griswold (1958) travailla sur la transmission de *Ceratocystis fagacearum* ("Oak wilt fungus") par *Drosophila melanogaster*.

Butler (1961) et Butler et al. (1963) mirent en évidence la transmission mécanique de la pourriture de la tomate en Californie par *Drosophila melanogaster*.

Glass et al. (1984) travaillèrent sur la vection de *Moraxella bovis* Hauduroy, responsable de la "kératoconjonctivite infectieuse des bovins", par les régurgitats de *Musca autumnalis* DeGeer. Ces auteurs conclurent que la régurgitation sur les yeux des veaux était le mode principal de transmission de la maladie.

Jefferies et al. (1986) émirent l'hypothèse de la transmission de *Leishmania chagasi*, responsable de la "leischmaniose" par les régurgitats de *Phlebotomus papatasi*. Cette hypothèse était basée sur les travaux de Parrot et al. (1952), Bray (1974), Lainson et al. (1977) et Molyneux (1977).

Drew et Lloyd (1987) envisagèrent la possibilité pour *Rhagoletis pomonella* d'absorber les levures et bactéries présentes à la surface des feuilles.

Louis et al. (1989), travaillant sur la vection de *Rhizopus stolonifer* par *Drosophila melanogaster*, exprima l'hypothèse, ici confirmée, que des mouches des fruits comme *Ceratitis capitata* pouvaient être des vecteurs d'agents pathogènes.

Les résultats obtenus doivent être interprétés comme autant "d'indices" nous amenant à une conclusion précise.

C'est ainsi que l'étude des rythmes de régurgitation et de réabsorption montre que ces deux comportements complémentaires sont à leur maximum dans les premières heures d'éclairement, période durant laquelle le rythme d'activité général de l'insecte est aussi maximum (Dridi, 1990). D'après nos observations dans les vergers, cette période correspond à une phase de prospection intense durant laquelle les insectes se déplacent d'un fruit à un autre, d'un arbre à un autre. Les observations menées en laboratoire ont permis de mettre en évidence la possibilité pour un individu de réabsorber les régurgitats d'un autre individu, augmentant d'autant plus le potentiel de dispersion des liquides régurgités.

L'étude des quantités de solution nutritive absorbées par un insecte nous a montré que le volume moyen ingéré était de l'ordre de 2,5µl, autorisant éventuellement le "transport" en solution d'un grand nombre de germes.

L'étude des potentialités de transmission a prouvé que la Mouche méditerranéenne des fruits est capable de transmettre un agent pathogène de végétal comme *Rhizopus stolonifer*, par voie externe ou interne. Le mode de transmission interne, mettant en jeu un transit partiel (régurgitation) ou total (feces) par le tractus digestif, peut être défini comme une transmission "semi-persistante" selon la conception de Harris (1980). Cette transmission a une plus grande importance qu'une simple transmission aléatoire, les mouches se posant pour se nourrir sur des parties du fruit où la cuticule est endommagée, les spores sont ainsi véhiculées jusqu'à proximité de la zone où elles pourront se développer.

L'association de l'ensemble de ces résultats prouve que, par le comportement de régurgitation, la Mouche méditerranéenne des fruits peut jouer un rôle important dans la transmission (par voie interne ou externe) et la dissémination (par dépôt de régurgitats infestés sur des fruits sains, d'un arbre à un autre, ou d'un verger à un autre) d'agents pathogènes de végétaux cultivés.

Ces résultats associés à la possibilité de "résurgence" des populations de ce ravageur dans des régions tempérées d'où on le croyait disparu (Cayol et Causse, 1993), doivent nous conduire à reconsidérer à la hausse l'impact possible de cette "Mouche des fruits d'importance économique".

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MECANISMES DE TRANSMISSION DE CHAMPIGNONS DE POURRITURE DES FRUITS PAR DROSOPHILES

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MECHANISMS OF TRANSMISSION OF FRUIT ROTS BY DROSOPHILAS

SUMMARY

Fruit-fly (Drosophila) may play an important role in fuit and grape rot transmission and epidemiology:

- rot fungi are efficiently disseminated by drosophila flies,
- germination of spores of several fungi species in Drosophila crop has been observed. This germination is followed by mycelium development, invasion of the crop, micro-sclerote differentiation. No apparent effects on insect fitness have been observed.
- Sporulated fungi have a selective attractivity and appetitivity for drosophilas
- live spores are disseminated by drosophila feces and possibly by regurgitation
- Wild rot fungi have been recover from the crop of drosophilas captured in the field
- There is an effective rot transmission by drosophilas in citrus orchards and fruit packing units.

It is thus confirmed that Drosophila can act as a fruit fly of economic importance causing a mechanical and possibly a multiplicative transmission of fruit rots.

KEY-WORDS:

Transmission of phytopathogenic microorganisms, fruit, grape, drosophila, digestive tract, gut, crop, microbiology, aseptic rearing, *Drosophila melanogaster*; fruit fly; fruit rot, *Monilia, Rhizopus, Penicillium, Cladosporium, Botrytis, Phoma, Geotrichum*, semi-persistant vector, circulative transmission.

RESUME:

Il est montré dans cet article:

- la réalité de la dissémination d'agents de pourriture des fruits et raisins par les drosophiles
- la germination de spores de différents espèces fongiques dans le jabot des drosophiles, suivie par le développement d'un mycelium, l'invasion du jabot, la différencition de micro-sclérotes, sans effet apparent sur la longévité de l'insecte
- l'attractivité et l'appétivité sélectives de souches fongiques sporulées sur les drosophiles,
- la transmission de spores vivantes par les feces des drosophiles et la transmission possible par régurgitation
- la présence de souches fongiques sauvages dans le jabot de drosophiles capturées dans la nature
- la transmission d'agents de pourritures des agrumes en vergers de Citrus et en stations de conditionnement

Ainsi, il se confirme que la drosophile peut être considérée comme une mouche des fruits d'importance économique, responsable de la transmission de champignons de pourriture des fruits au moment de la récolte.

INTRODUCTION

Le rôle des insectes, et notamment des diptères, dans la vection et la dissémination des pathogènes fongiques de végétaux, est encore mal connu, bien que fortement soupçonné (ZITTER & TSAI 1980). C'est particulièrement le cas pour les champignons de pourriture des fruits se développant avant ou après récolte (AGRIOS 1980)

Précédemment, nous avons mis l'accent sur l'importance des drosophiles en tant que vecteur/disséminateur de germes pathogènes aux fruits (LOUIS et al.1989). Nous avons mis en évidence le transport de spores de champignons, notamment du genre Rhizopus, en vergers et

dans les salles de conditionnement des fruits. Nous avons présenté les premiers éléments sur le mécanisme de transmission chez *Rhizopus stolonifer*: adhésion des spores sur la cuticule, absorption de spores *per os*, passage dans le tractus intestinal et stockage dans le jabot, où leur germination a été observée.

Le présent article élargit la recherche à d'autres espèces de champignons de pourriture des fruits et montre notamment que les spores qui se sont introduites dans le jabot, après avoir germé, poursuivent leur développement en un mycelium qui envahit cet organe et peut différencier un microsclérote. Il est montré que les spores rejettées par les feces sont toujours vivantes. L'attractivité et l'appétitivité de différentes espèces de champignons pour les drosophiles sont comparées. Plusieurs espèces de champignons sont cultivées à partir de jabots de drosophiles collectées dans la nature.

MATERIEL ET METHODES:

1. Souche de Laboratoire de D. melanogaster:

Souche Champetières "germ-free" à phénotype sauvage hautement stabilisé par une quarantaine d'années de croisement frère x soeur. Les individus utilisés sont âgés de 2 ± 1 jours. L'élevage de référence est mené en conditions axéniques (DAVID, 1959). Les insectes expérimentaux sont élevés sur milieu stérile adapté sans benzoate de méthyle afin de ne pas inhiber les champignons. Ces conditions obligent à respecter rigoureusement l'aseptie. Pour éviter les confusions entre levures entières et conidies de champignons en observation en microscopie photonique, les levures sont remplacées par un poids équivalent d'extrait de levure pour bactériologie.

Milieu modifié (milieu "CL" sans levures): 250 ml d'eau distillée, 25 g de saccharose, 3g d'extrait de levure, 4g de gélose, 0,1g de chlorure de sodium, stérilisation à l'autoclave.

Sauf précision contraire, la température d'élevage est 25°C.

2. Souches de champignons:

Quelle que soit la souche, les spores ou les conidies sont désignées indifféremment sous le terme de "spores".

-Station de Technologie des Produits végétaux, INRA Avignon: souches de *Monilia fructigena, M. laxa* et *Rhizopus stolonifer*.

-Station de Pathologie Végétale INRA de Bordeaux: souches *Botrytis cinerea* C1 isolée de Champagne ; souches B1 et S22 isolées du vignoble bordelais

-Station de Pathologie Végétale INRA d'Angers: *Phoma herbarum, Alternaria alternata*

La mise en culture de parties de tubes digestifs de drosophiles de la nature a permis d'obtenir trois isolats: *Penicillium expansum* dénommé G1, *Cladosporium herbarum* (G2) et *Geotrichum candidum* (G3).

3. Essais d'attractivité en Laboratoire: voir fig 1

Les expérimentations se font dans l'obscurité totale, les drosophiles étant très photophiles. Des essais préliminaires ont montré une répartition aléatoire des insectes dans les différents enclos sans attractifs. Après chaque expérience le dispositif est nettoyé à l'hypochlorite de sodium à 3° chlorimétrique, rincé abondamment et séché. La durée de chaque expérience est de six heures. Les attractifs sont des milieux de culture avec des colonies de champignons au même stade de développement (7 répétitions).

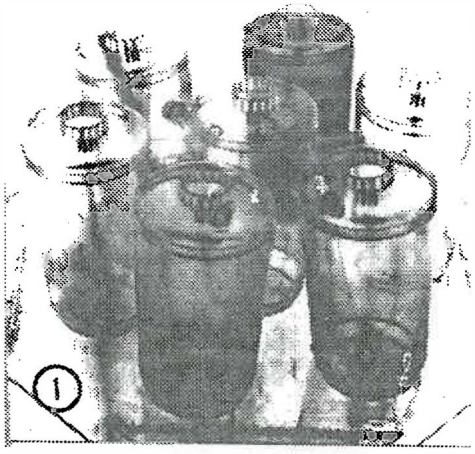
4. Essais d'appétence: Des adultes de drosophile sont placés pendant 15H sur une culture de champignon sporulée et leur consommation de spores est estimée par dissection du tube digestif et examen microscopique.

RESULTATS

1. ATTRACTIVITE ET APPETENCE COMPAREES DE PLUSIEURS SOUCHES DE CHAMPIGNONS:

1.1. Attractivité comparée:

Les résultats montrent une attractivité préférentielle nette pour *Phoma herbarum*



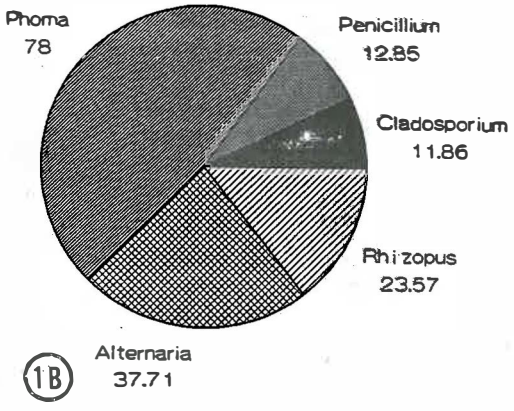


FIGURE 1 Cages à migration: *L'enceinte centrale reçoit les drosophiles. Les enceintes périphériques contiennent les souches de champignons cultivées sur gélose en boîtes de Petri. Un conduit relie l'enceinte centrale à chacune des enceintes périphériques.*
FIG.1B *Attractivité comparée de différentes espèces de pourritures des fruits.*

1.2. Appétence:

Dans la plupart des cas, la consommation des spores est irrégulière et concerne environ la moitié des individus. Par contre, dans le cas de *P. expansum* G1, la totalité des insectes se "gavent" littéralement de spores de ce champignon, qui distendent leur tube digestif (Fig.2). Il y a donc une très forte appétence pour *P. expansum* G1.

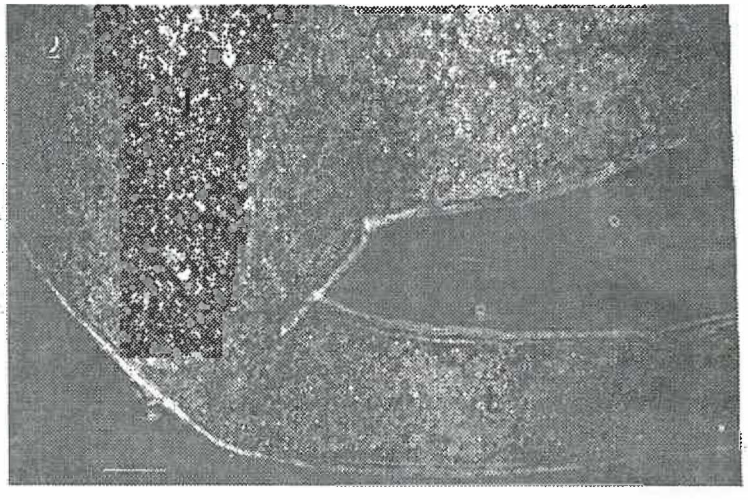


FIGURE 2. Drosophile alimentée sur une colonie sporulée de Penicillium expansum*: le jabot (j) et le tractus (t) sont complétement remplis de spores.* Barre=10 µm

2. DISSEMINATION DES CHAMPIGNONS DE POURRITURES PAR DROSOPHILES:

2.1. Passages successifs en tubes:

La méthode de transfert dans des tubes stériles successifs est habituellement utilisée pour débarrasser les insectes de leurs contaminants extérieurs. On considère en général qu'après six passages, les insectes sont débarrassés des contaminants, notamment par leur comportement de toilettage (N. PLUS, communication personnelle).

Des adultes âgés de un jour sont élevées pendant 48h sur milieu sans levures entières ni benzoate de méthyle pour éliminer cet antifongique de leur organisme. Après une diète hydrique de 8h, ils sont élevés pendant 12h sur une culture de *B. cinerea* (souche B1), séparées en un lot de 10 mâles et un lot de 10 femelles et passées dans des tubes successifs sans fongicide toutes les deux heures pendant 24 heures, puis tous les deux jours, pendant 22 jours en tout.

La croissance du champignon est constatée dans l'ensemble des tubes ayant servi à l'élevage des femelles jusqu'au seizième jour, puis les tubes des six derniers jours sont restés stériles. Chez les mâles, les tubes sont restés stériles dès le 12° jour.

2.2. Examen de la cuticule par microscopie électronique à balayage (MEB):

Nous avons précédemment montré par MEB la présence de spores sur le corps de l'insecte, après deux passages successifs dans des tubes de nettoyage (LOUIS *et al.* 1989). Au quatrième passage, il est encore possible de déceler quelques spores. Nous n'en avons plus retrouvé chez des insectes ayant subi six passages successifs.

2.3. Transit par le tube digestif:

Cette série d'expériences a pour but de préciser si des spores de champignons ingurgitées traversent effectivement le tube digestif sans être digérées, et si elles conservent leurs capacités germinatives.

2.3.1. Passage de spores par le tube digestif:

Après avoir été alimentés sur cultures sporulées, les insectes sont disséqués et le contenu du tube digestif postérieur, notamment de son extrémité distale (rectum) est examiné entre lame et lamelle.

Chez les individus ayant absorbé des spores, celles-ci sont retrouvées en grand nombre dans le tube digestif postérieur et dans le rectum dans le cas de *Rhizopus stolonifer*, *Botrytis cinerea, Monilia fructigena* et surtout *Penicillium expansum* G1.

2.3.2: Potentiel germinatif des spores rejetées avec les feces:

Le potentiel germinatif de spores contenues dans la partie distale du tube digestif d'insectes alimentés pendant une journée sur culture sporulée, est estimé chez *P. expansum* de deux manières:

2.3.2.1. Prélèvement stérile et mise en culture du rectum sur un milieu de culture gélosé: La croissance de colonies de *P. expansum* a été constatée.

Cette méthode n'est pas rigoureuse, la présence de spores provenant de l'extérieur des insectes n'étant pas exclue malgré les précautions prises. Une méthode d'examen direct a donc été utilisée.

2.3.2.2. Prélevement de l'extrémité postérieure du tube digestif, montage entre lame et lamelle dans du liquide de Ringer stérile et observations successives au microscope à contraste de phase. La quasi-totalité des spores arrivées dans le rectum gonflent au bout de 24 heures et émettent un tube germinatif. Certains tubes germinatifs arrivent même à sortir par la section du rectum ou par l'anus (fig. 3). Cette méthode, essayée sur *P. expansum*, a été étendue avec succès à d'autres souches, dont *R. stolonifer* et *B. cinerea*.

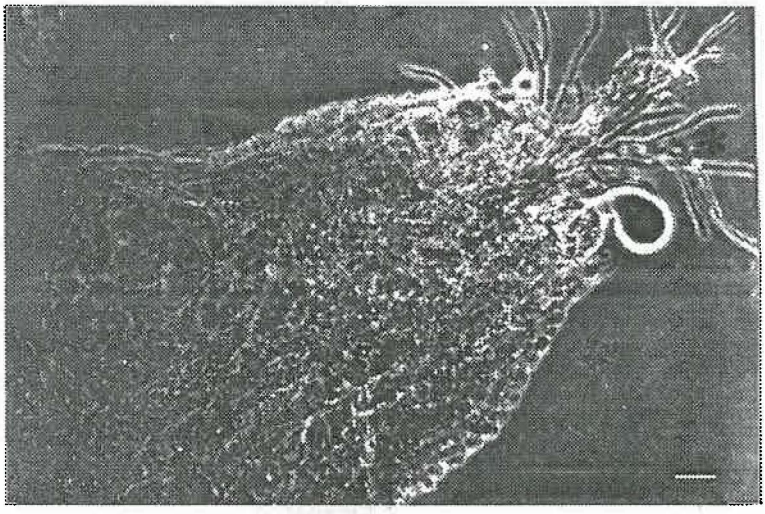


FIGURE 3:
Une drosophile est alimentée pendant une journée sur une culture sporulée de P. expansum. Son rectum est disséqué et placé entre lame et lamelle dans de l'eau pendant 24 heures: la germination in vitro des spores et le développement d'hyphes myceliens (*) démontre que ces spores étaient vivantes. Barre=10 µm

3. PASSAGE DES SPORES DANS LE JABOT:

Le jabot, diverticule du tube digestif antérieur, peut, chez les drosophiles prélevées dans la nature ou élevées non aseptiquement, renfermer des levures et (ou) des bactéries vivantes en quantités variables, ainsi que des champignons.

3.1. Recherche de champignons dans le jabot de drosophiles de la nature:

Des drosophiles capturées sur des écarts de triage sont disséquées après 4h de diète. Leur jabot examiné en microscopie en contraste de phase contient un mycelium en cours de développement chez 9 femelles sur 20. Le champignon est en général peu développé, sauf dans deux cas où il a envahi tout le jabot et gagné le tractus digestif. Dans tous les cas les bactéries sont abondantes et des levures sont présentes dans 14 cas.

Sur 15 mâles disséqués, du mycelium (en début de développement) est présent dans 10 jabots. Des bactéries sont présentes dans l'ensemble des cas et des levures dans 9 des cas.

Plusieurs individus prélevés en vergers sont disséqués stérilement après désinfection externe du corps à l'hypochlorite de sodium, et le tube digestif est mis en culture. Trois isolats sont obtenus: *Penicillium expansum* (G1, pour "Gotheron 1"), *Cladosporium herbarum* (G2) et *Geotrichum candidum* (G3)

3.2. Suivi en laboratoire des spores localisées dans le jabot:

Les observations précédentes et des données anciennes concernant les levures (SEMICHON, 1906) nous ont incité à rechercher et à suivre l'évolution des spores de champignons de pourriture de fruits dans le jabot après leur ingestion par l'insecte.

Les insectes sont des "Champetières" aseptiques élevées sur milieu sans levures, alimentées sur culture sporulée de champignons puis replacées sur milieu aseptique sans levures. Les jabots sont prélevés à intervalles de temps donnés et examinés en contraste de phase. L'étude porte sur *Monilia laxa, M. fructigena, R. stolonifer, B. cinerea* souches B1, C1 et S22, *P. expansum, C. herbarium.*

Des spores se retrouvent dans une proportion variable de jabots, alors que la présence de filaments mycéliens en provenance directe du milieu n'a jamais été observée dans le jabot ni dans le tube digestif.

3.2.1. Germination et développement d'hyphes mycéliens dans le jabot:

Comme nous l'avons montré pour *R. stolonifer* (LOUIS et al., 1989), dans toutes les souches étudiées (sauf chez *B. cinerea* S22) le gonflement puis la germination de spores avec émission d'un filament germinatif a été constaté.

Le développement de tubes germinatifs en hyphes mycéliens est ensuite constaté, en général en moins de 24 h, pouvant aboutir à l'envahissement complet du jabot.

A partir du moment où un peloton mycélien est constitué dans le jabot, la croissance des filaments se poursuit en périphérie du peloton, alors qu'au centre les filaments ont tendance à dégénèrer (fig 4), comme cela est connu de la croissance des colonies fongiques.

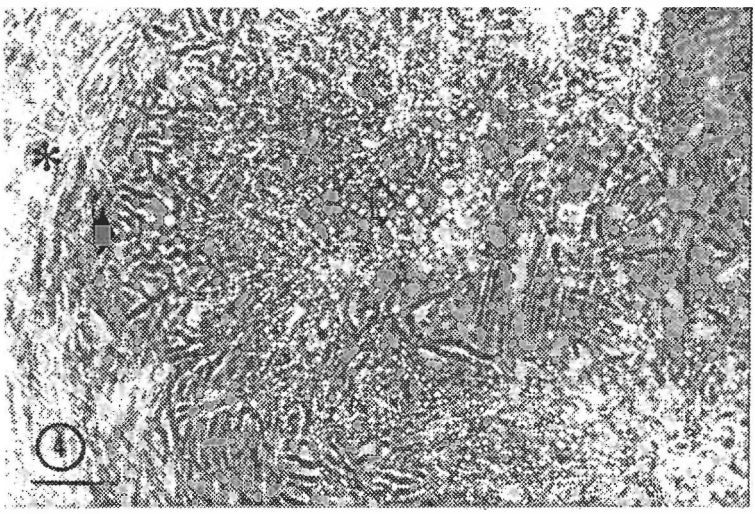


FIGURE 4: Peloton mycelien contenu dans le jabot de D. melanogaster *(contr. phase). * paroi du jabot, ▶ filament en cours de croissance en périphérie de la colonie, ▷ mycelium vacuolisé.* Barre= 10 µm

Exceptionnellement, la sortie du mycélium hors du jabot dans le canal qui relie le jabot à l'oesophage et la présence de filaments mycéliens dans le tractus digestif peuvent être constatées. Par contre, il n'est pas observé d'invasion des organes de la mouche ni d'action pathogène similaires à celles décrites par FROBISHER (1926).

3.3. Etude quantitative comparée du développement de trois souches de *B. cinerea* dans le jabot:

3.3.1.: *B. cinerea* souche B1:

Les mâles et les femelles sont séparés dès leur émergence en deux groupes d'environ 100 individus chaque. Ils sont alimentés pendant une journée sur une culture sporulée de *B. cinerea* souche B1 puis transférés dans des tubes stériles qui sont changés tous les deux jours. La présence de spores dans certains jabots est constatée par dissection. Leur germination débute les 3° ou 4° jours. Le développement d'un peloton mycélien est constaté au bout de cinq jours. Les insectes survivants sont conservés en élevage jusqu'au 30° jour suivant le début de l'expérience, et sont disséqués en totalité. 31 femelles sur 74 (42%) présentent alors du mycelium dans leur jabot, contre 8% de mâles (2 sur 26).

3.3.2. *B. cinerea* souches S22 et C1:

Dans les mêmes conditions que ci-dessus, aucun développement de mycelium n'est constaté chez les deux sexes pour S22. Pour C1, aucun mycelium n'est retrouvé chez les males, et environ 20% des femelles présentent du mycelium dans leur jabot.

3.3.4. Influence de la température d'élevage: Différenciation de "microsclérotes" de *B. cinerea* (souche B1) dans le jabot de *D. melanogaster*:

Les températures élevées ne sont pas favorables aux champignons, une germination à 25°C provoquant la formation d'un mycelium stérile chez *B. cinerea*. Les températures plus basses semblent plus favorables, 18°C étant l'optimum de germination, 15°C l'optimum de formation des sclérotes. Pour nous rapprocher des températures optimales pour le champignon tout en conservant une certaine activité aux insectes, nous avons choisi d'élever les insectes à 20°C pendant une semaine, puis à 17°C pendant trois semaines. Des lots de 20 mâles et de 20 femelles sont placés pendant un mois à 20°C, à 17°C, ou à 20°C pendant 8 jours puis à 17°C jusqu'à la fin de l'expérience.

Les résultats sont voisins de ceux obtenus à 25°C (près de 40% des femelles et quelques mâles contiennent un peloton mycélien dans leur jabot). Notamment, chez deux femelles un mycelium très nettement modifié est observé, avec différenciation d'un amas mycélien en coquille de couleur brune formé de filaments de petite taille très serrés les uns contre les autres (fig 5). Ces formations semblent constituer une différenciation de type sclérote (microsclérote).

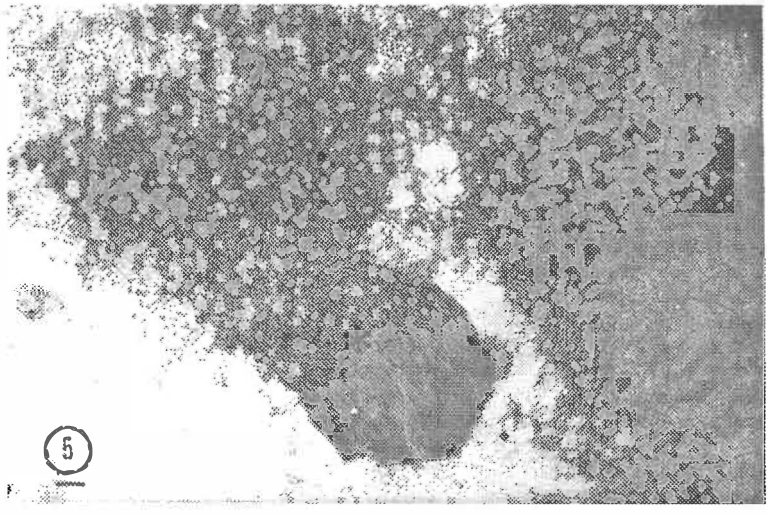


FIGURE 5. Botrytis cinerea *infectant un jabot de* Drosophila melanogaster. *Mycelium différencié en microsclérote * (infection agée de un mois).* Barre= 10 µm

4. TRANSMISSION POTENTIELLE PAR REGURGITATION:

La régurgitation de suc alimentaire est connue chez les diptères (GRAHAM-SMITH 1930, HEINDRICHS *et al.* 1992, HEINDRICHS *et al.* 1993). Elle vient d'être démontrée chez la mouche méditerranéenne des fruits *Ceratitis capitata* par CAYOL *et al.* (1993). Nous avons recherché l'existence de ce phénomène chez la drosophile, en vue de mettre en évidence une éventuelle régurgitation de spores.

Nous n'avons pas pu observer, comme chez les deux espèces précédentes, de régurgitation d'une sphère de liquide qui est ensuite réingérée après évaporation partielle.

Un procédé d'observation particulier (GRAHAM-SMITH 1930) est utilisé pour repérer les excreta et les traces de trompe sous microscope à contraste de phase.

Des lames de verre sont recouvertes de gélose à 1,8% stérile. Les drosophiles sont placées 8h en condition de diète hydrique puis sont alimentées pendant 12 heures sur milieu stérile sans levures additionné de bleu de méthylène. Ensuite les mouches sont soumises à nouveau à la diète, puis placées séparément sur une lame gélosée, qui est examinée trois heures après.

Les excreta forment des taches bleutées plus ou moins granuleuses. Cela montre que en 15 heures une partie du bleu de méthylène a parcouru la totalité du tractus intestinal.

Les traces de trompes sont de deux types: nettes ou diffuses (fig. 6).

Les traces nettes forment une impression en creux dans la gélose, où se distinguent les détails anatomiques des labelles. Elles nous semblent correspondre à des phases d'aspiration de la trompe.

Les traces diffuses sont en léger relief, sans détails anatomiques. Elles renferment des grains de bleu de méthylène. Elles correspondent vraisemblablement à des phases de régurgitation de liquide en provenance du tube digestif.

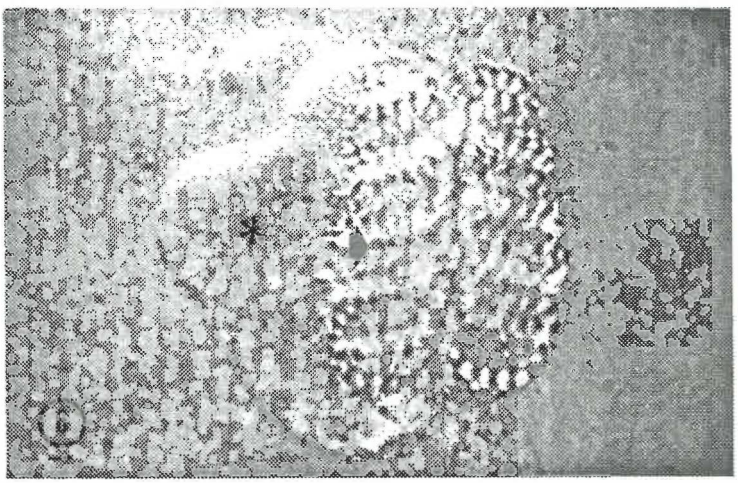


FIGURE 6. Double trace de trompe de D. melanogaster *sur gélose. Trace lisse (*): régurgitation, et empreinte des labelles ( ): ré-aspiration.* Barre= 10 µm.

Le transport d'éléments figurés après 15 h de transit intestinal et par régurgitation étant confirmé, nous avons tenté de montrer la régurgitation de spores de champignons, mais sans succès jusqu'ici.

5. TRANSPOSITION DES RECHERCHES CI-DESSUS EN VERGERS D'AGRUMES:

Certains des protocoles expérimentaux décrits ci-dessus ont été repris en vergers et stations de conditionnement d'agrumes dans la région du Souss (Agadir, Maroc) et leurs résultats feront l'objet d'une publication détaillée ultérieure.

Sommairement, la présence de drosophiles a été montrée en vergers et stations de conditionnement, leurs effectifs variant au cours de l'année. Les champignons agents responsables des principales pourritures des agrumes (*Penicillium italicum, P. digitatum* et *Geotrichum candidum*) ont été isolés de drosophiles piégées. Il a été montré que *G. candidum*, agent de la pourriture amère, était rarement isolé de l'air ambiant, mais par contre était isolé de la plupart des drosophiles analysées. D'autre part, *G. candidum* est l'agent de pourriture le plus attractif pour les drosophiles.

DISCUSSION GENERALE:

Les observations sur le terrain avaient montré (LOUIS *et al.* 1989) que les drosophiles se déplacent au cours de la journée et explorent successivement les fruits, pourris ou non, tombés au sol et les frondaisons, y compris les fruits, mûrs ou non. Ces données comportementales sont d'ailleurs en accord avec les résultats de piégeages et d'observations en dehors de vergers, relatés de biotopes divers (ROCHA-PITE 1978). *M. laxa* est surtout redouté en vergers, alors que *M. fructigena, R. stolonifer, B. cinerea, P. expansum, C. herbarium* se développent surtout après récolte. Quant à *B. cinerea*, il est encore plus redouté comme agent de pourriture des raisins que des pêches.

Les essais d'attractivité et d'appétitivité montrent que la souche de champignons la plus attractive est *Phoma herbarum*, la plus consommée est *P. expansum* (G1). Attractivité et appétence sont vraisemblablement liés à deux facteurs différents. PATERSON *et al* (1987) ont montré que certaines souches répertoriées à l'IMI de *P. expansum* pouvaient être toxiques pour la drosophile, et que les métabolites secondaires de *P. expansum* étaient répulsifs pour la drosophile, sauf la patuline qui ne différait pas du témoin. La variabilité entre souches doit donc être très grande. Les champignons de pourriture les plus redoutés par les producteurs, Monilia, Rhizopus et Botrytis ne sont pas ceux qui ont eu le plus d'effet sur les drosophiles testées. Il s'agissait cependant de souches de Laboratoire, alors que G1 avait été isolée de drosophiles de la nature peu avant le test d'appétence. Ceci pourrait signifier que G1 ait été particulièrement adaptée à la consommation par drosophiles. Dans tous les cas, c'est bien l'absorption de spores qui a été constatée, mais non celle de mycelium.

Les expériences en cage comportementale et les passages successifs sur tubes stériles confirment que les drosophiles sont, globalement, des vecteurs efficaces de spores de champignons de pourriture des fruits.

La microscopie électronique à balayage a mis nettement en évidence la présence de spores de plusieurs espèces de champignons sur la cuticule de l'insecte, puis leur disparition progressive au cours des passages en tubes stériles. Le fait que après six passages il n'ait plus été retrouvé de spores confirme les données de la littérature, mais n'est pas une preuve absolue de la disparition totale des spores, qui peuvent subsister dans des endroits inaccessibles à l'observation et rester potentiellement contaminantes.

Le passage de spores par le tube digestif et la conservation de leur potentiel germinatif sont démontrés sans ambiguïté par plusieurs méthodes convergentes. La potentialité de régurgitation est confirmée, mais la régurgitation de spores n'a pas pu être démontrée.

Une des données les plus significatives de ce travail nous semble être la démonstration du stockage des spores dans une zone particulière du tube digestif, le jabot, suivi de leur germination et de leur développement en un mycelium qui peut persister et poursuivre son développement pendant au moins un mois. Le mycelium est généralement considéré chez les champignons comme une forme de résistance. Le développement de pseudosclérotes, autre forme de résistance, à température relativement basse, est une troisième forme de conservation de l'inoculum. Cela montre que les drosophiles infectées restent une source potentielle d'inoculum, sous trois formes différentes (spores, mycelium, pseudosclérotes) qui pourrait jouer un rôle non seulement en période de production de fruits, mais encore en dehors de cette période, notamment lors du passage de la mauvaise saison où les drosophiles subsistent au stade adulte (MATOS et al. 1987), et où certaines restent porteuses de microorganismes (virus sigma, FLEURIET 1981).

En ce qui concerne le développement d'hyphes mycéliens dans le jabot, de grandes différences apparaissent d'un genre ou d'une espèce de champignon à l'autre, mais il est remarquable que des différences aussi importantes se révèlent entre souches d'une même espèce, telle *B. cinerea*. La souche B1 se développe largement dans le jabot des femelles, et peu chez les mâles. La souche S22 ne se développe pas du tout dans le jabot, aussi bien des mâles que des femelles. La souche C1 présente un cas intermédiaire, en se développant uniquement chez les femelles, mais dans peu de cas seulement. Les facteurs de spécificité en jeu doivent faire intervenir des différences quantitatives dans la consommation de spores entre sexes, et des différences d'appétence et (ou) de potentiel germinatif entre souches de *B. cinerea*. La température d'élevage,

fixée ici *a priori* à 25°C, peut également jouer un rôle sur les caractères biologiques des souches de champignons en freinant leur développement. C'est pourquoi des études à températures plus basses ont été abordées. Elles ont permis de montrer la différenciation de microsclérotes, forme de résistance particulière.

Les résultats obtenus lors des passages successifs sur milieux stériles, qui aboutissent à une perte de transmission après environ deux semaines, semblent en contradiction avec la persistance du champignon dans le jabot sous forme de mycelium ou de pseudosclérote. Cela peut s'expliquer par la rareté ou l'absence de sortie du champignon hors du jabot, phénomène que nous n'avons observé que très rarement.

Les résultats préliminaires obtenus sur les relations drosophiles/agrumes/champignons de pourritures dans le Souss indiquent que la drosophile est un agent de transmission des germes pathogènes aux agrumes, notamment de l'agent de la pourriture amère, *G. candidum*, qui n'est pas véhiculé par l'air mais est largement transporté par les drosophiles.

Il est confirmé que les drosophiles sont des vecteurs effectifs de champignons de pourriture des fruits, par dissémination active de spores. En suivant la terminologie utilisée surtout pour les homoptères vecteurs (HARRIS et MARAMOROSCH 1980), on peut considérer que les drosophiles sont des agents de "vection non-persistante" par dissémination immédiate de spores externes. Elles sont potentiellement agents de "vection semi-persistante" par stockage de spores dans le jabot, et même de "vection persistante (multipliante)" par le développement de mycelium et de pseudosclérote dans le jabot. Il reste cependant à démontrer que cette possibilité a un rôle effectif, et comment.

Le comportement exploratoire systématique des drosophiles sur fruits à différents stades de maturité en font des "vecteurs à tête chercheuse" plus efficaces que le vent, considéré jusqu'ici comme le vecteur principal de champignons de pourriture des fruits en vergers. L'action des drosophiles -et sans doutes d'autres insectes- se poursuit ensuite, lors de la conservation et de la commercialisation des fruits. Après récolte, et notamment en salles de conditionnement, une hygiène rigoureuse est indispensable pour éviter les pullulations. C'est pourquoi il nous semble important, en accord avec AGRIOS (1980) que soient poursuivies les études sur le rôle des insectes comme vecteurs de champignons de pourriture des fruits.

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Session 4

S.I.T. Sterile Insect Technique

Chairman: J. PIEDADE-GUERREIRO

Secretaries: J. HENDRICHS
P. LIEDO
A. WAKID

Report on Session 4

J. PIEDADE-GUERREIRO; J. HENDRICHS; P. LIEDO; A. WAKID.

Important achievements have been made during the past years to better understand and improve the SIT; This WG recommends increased support to SIT, both in research and applications; New development such as the application of genetic sexing and more in depht-economical analysis have shown that SIT have more applications than those previosly though, specially if environmemtal costs are considered.

EFFECT OF GAMMA IRRADIATION AND DIMETHOATE INSECTICIDE ON FEMALE FECUNDITY AND ADULT SURVIVAL OF THE MEDFLY, *CERATITIS CAPITATA* (WIED.)

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Summary

Laboratory experiments were conducted to study the effect of gamma irradiation (50, 70, and 90 Gy) applied to the pupal stage of the medfly, *Ceratitis capitata* (Wied.), and a treatment with dimethoate insecticide (0.93 ppm) applied to the adults on female fecundity and the percent males and females surviving more than 15 days. When normal or treated females were inseminated with normal,irradiated or treated males, egg production was not significantly affected. However, when irradiated females were inseminated with normal or treated males, oviposition was completely inhibited. Survival of treated males was highly affected, while female survival was insignificantly affected. However, irradiation did not significantly affect survivals of males or females. There were insignificant differences in survivals between treated and irradiated males at 50 and 70 Gy, while at 90 Gy there was a clear effect.

1-Introduction

As a result of the use of chemicals before releasing gamma sterilized insects, the treated area after release will contain different types of insect populations according to the degree of sensitivity of individuals to irradiation or insecticidal stress, and to the response of the progeny of partially affected individuals when mated with the released insects. Hence, all the different kinds of insects may or may not mate and subsequently new generations are expected as a result of the different matings. The present study is apart of a research plan aiming at determination of the possible separate and combined effects of applying dimethoate as an

adulticide used against the medfly preceded by the pupal irradiation of the fly. In this paper, the effects of irradiation and dimethoate on female fecundity and adult survival are studied.

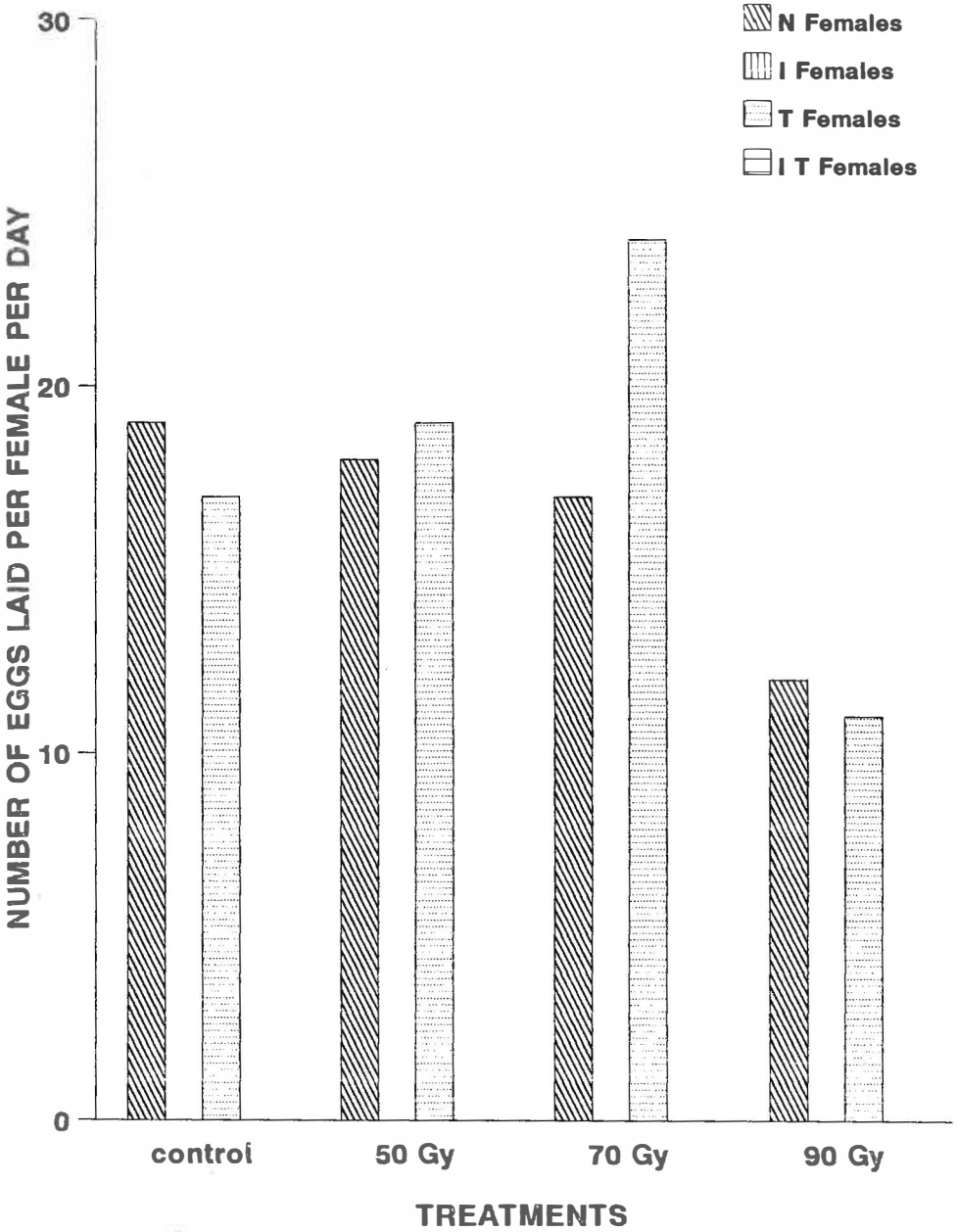
2-Materials and Methods

Adult medflies were fed on a mixture of yeast hydrolysate enzymatic and sugar (1:3). Larvae were reared in the laboratory on a synthetic medium of bran, yeast, molasses, Na benzoate and hydrochloric acid in water. The colony was kept at 25±2°C and 50-70% R.H. Irradiation of full grown pupae with 50, 70, or 90 Gy was performed using a gamma cell (Co-60). Dimethoate(40%) was used as a dry film applied to the adult stage with a concentration of 0.93 ppm. Female fecundity was assessed as number of eggs laid per female per day through 15 days. Adult survival was assessed as percentage of adults survived for at least 15 days after treatment with dimethoate or irradiation.

3-Results

The data illustrated on Figures (1 and 2) show that when one-day-old normal or treated females were inseminated with normal or irradiated males at 50, 70 or 90 Gy, the mean number of eggs laid per female per day was not clearly affected. On the other hand, when irradiated females were mated with normal, treated or irradiated males, no egg production was observed. This result is in agreement with those obtained by many authors (e.g. Hooper, 1971; Shoukry, 1974). However, the effect of some insecticides on the medfly was found to reduce egg production of the treated females. For example, Albrecht and Sherman (1987) reported that topical toxicity of avermectin B1 (abamectin) with doses that caused less than 25% mortality of female med flies significantly reduced fecundity whether or not males were treated before mating. Also, Chang et al. (1988) reported that when 7 benzyl 1,3-benzodioxole analogues were tested for female medflies, single topical doses of J-2581 at 3.0/µg/fly and J. 3263 at 1.0/µg/fly were found to cause a temporary delay in ovarian growth in females by 9 and 13 days, respectively. They found that oocyte growth and subsequent egg production and egg hatch were significantly reduced. These results are not in agreement with the present ones, likely due to difference in the type and concentration of the used insecticides.

The data recorded on the effects of gamma radiation and dimethoate on adult survival (Fig. 3 and 4) showed that there was a significant reduction in male survival by using dimethoate insecticide while irradiation with the tested doses had no significant effect on male survival. Moreover, neither dimethoate nor irradiation affected female



IG. (1): Effect of gamma radiation or treating with Dimethoate (0.93 ppm) on the fecundity of female Ceratitis capitata when mated with irradiated males (as pupae).

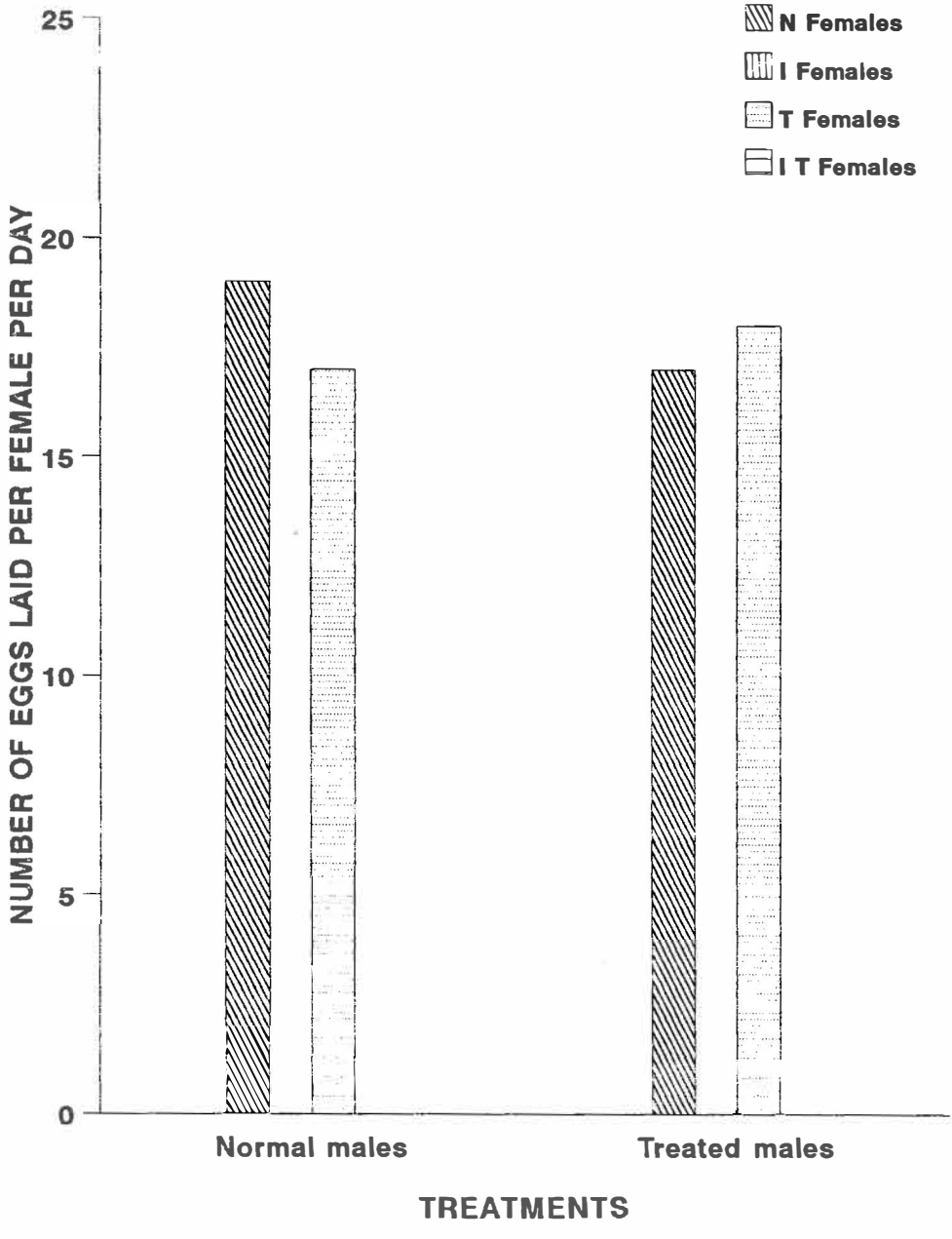


FIG. (2): Effect of gamma radiation or / and treating with Dimethoate on the fecundity of female Ceratitis capitata when mated with treated (Dimethoate) males.

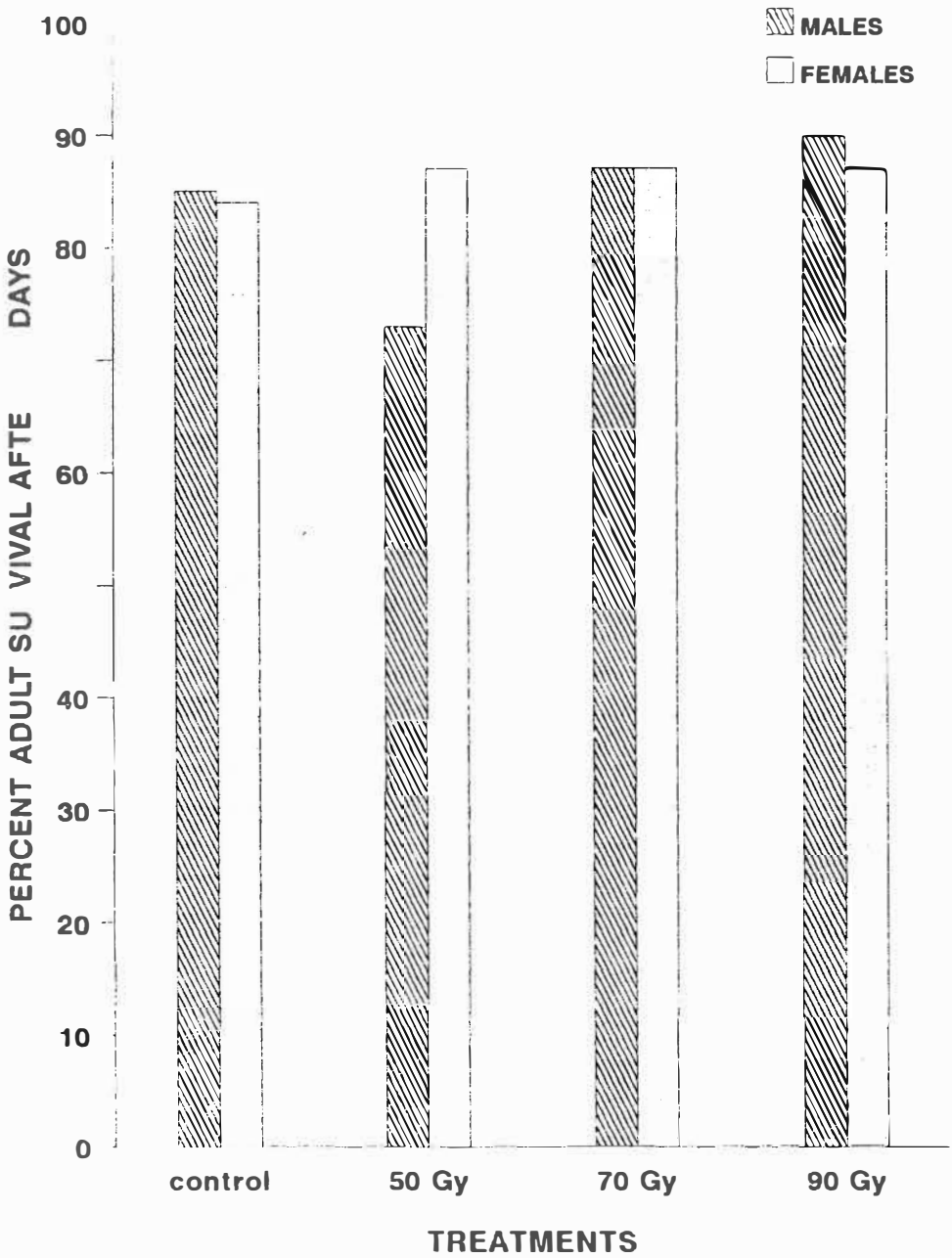


FIG. (3): Effect of gamma radiation (50, 70, 90 Gray) on % adult survival after 15 days of the Medfly, Ceratitis capitata

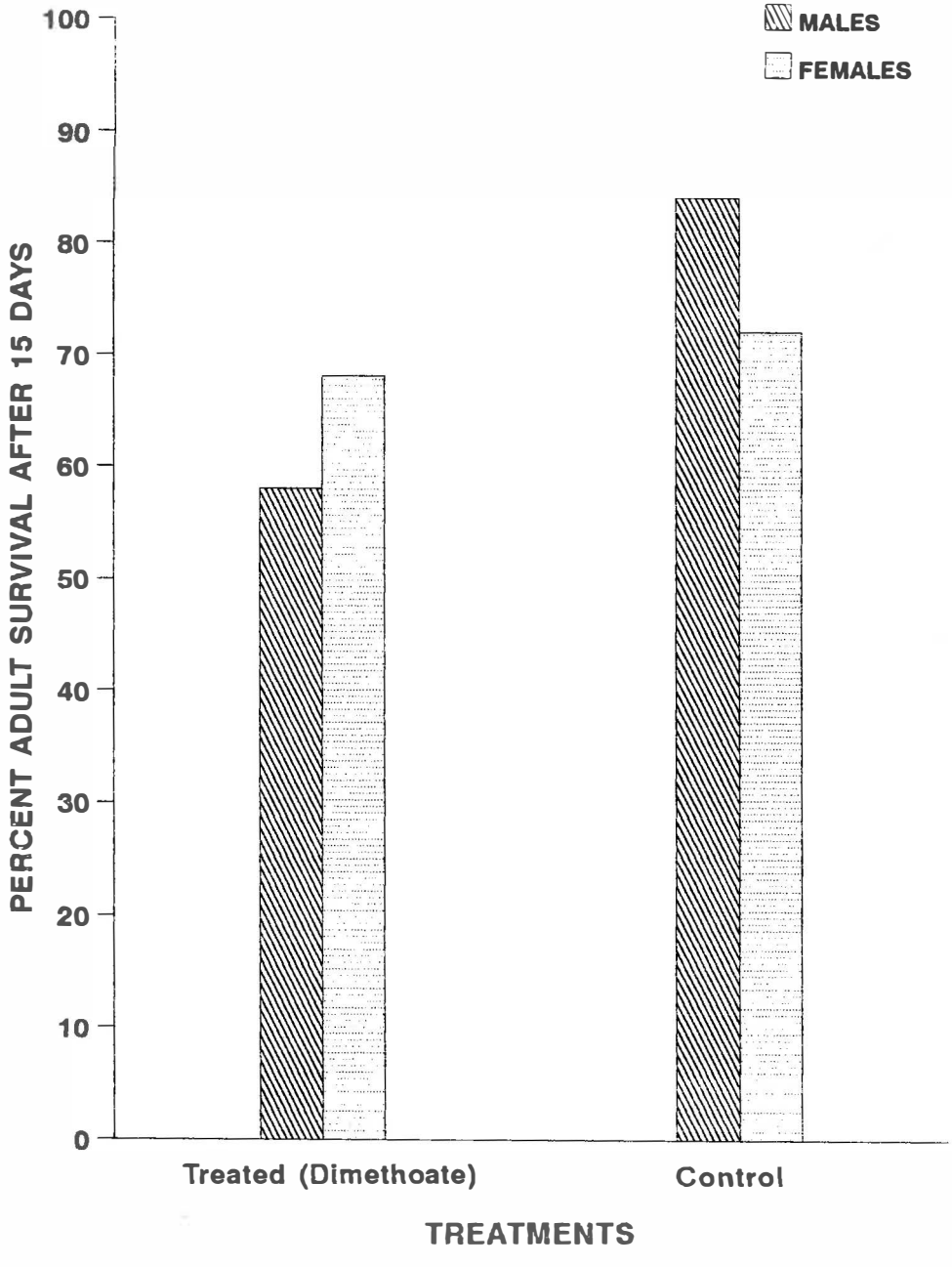


FIG. (4): Effect of treating the adult Ceratitis capitata with Dimethoate (0.93 ppm) in their survivals after 15 days.

survival. However, the comparison between the percentages survival of males and females after 15 days revealed insignificant differences between them whether they were normal or treated with dimethoate. The effect of irradiation on the medfly in the present studies are similar to most of authors working on the medfly (e.g. Feron, 1966; Fares and Awadallah, 1973; Wakid, 1973; Wakid et al., 1982) However, other reports indicated that high levels of radiation (more than 90 Gy) affected adult survival (e.g. Hooper, 1969; Zumreoglu and Akman, 1988). As to the effect of insecticides on the medfly survival few reports were available. However, Keiser et al., (1988) showed that male medflies lived longer than females treated with malathion.

4-Conclusions

The results obtained from the present and other related studies on the effects of gamma radiation and dimethoate insecticide showed two points. Firstly, they agree with those obtained by others in that gamma radiation drastically affects the male and female fertility but its effect on adult survival is limited. Secondly, regarding the insecticide effects when applied alone, there was a wide variation in results obtained by other authors mainly due to either the type of insecticide used or the concentration applied. However, the present results indicated that female fecundity was not affected by dimethoate application and that male survival was highly affected while females were not. These results may lead to the conclusion that when irradiated sterile adult flies are released in a field previously sprayed with dimethoate, their reproductive potential will not change in case they are mated with the still alive adults that escaped the insecticide toxicity. Generally these results show that suppression of the medfly population with a reasonable dose of dimethoate before SIT application will help the implementation without affecting the expected results.

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The Sterilization of Med Fly *Ceratitis capitata* Wied. with Neutronic Radiation

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Abstract

The Authors present the results of the use of neutronic radiation on the sterilization of the species *Ceratitis capitata* Wied.

This study was carried out by irradiating the pupae in the "Reactor Português de Investigação (RPI)", a pool type reactor with 1 MW nominal thermal power, using a core of enriched uranium. The water temperature in the irradiation position was about 28°C.

The neutronic radiation of the reactor has a wide energy spectrum, from thermal to fast neutrons, and is always associated with gamma radiation. Therefore two different types of neutronic radiation were used. In the first study the biological material was exposed to total neutronic radiation, composed of thermal neutrons, fast neutrons and gamma radiation. In the second study a cadmium filter was used to obtain a partial neutronic radiation, composed only of fast neutrons and gamma radiation. In each experiment 500 pupae with 6 days of metamorphosieal evolution were used.

Although differences in adult's sterilization have been observed according to whether total or partial neutronic radiation was used, the doses of neutronic radiation required to obtain the same degree of sterility in adults are two or three times lower as compared with those necessary using only gamma radiation. This is quite important in reducing the adverse effects in physiology and behaviour of the insects.

Introduction

If the autocidal control of a given species by sterilization of the males seems in theory to be simple, it is in practice a very complex matter.

The effect of ionizing radiations in the insects behaviour must be conditioned in a way as to obtain males that are not only sterile but also vigorous and sexually competitive, aiming for their sucess in the sterile male technique (HOOPER, 1970).

The main agent used in the sterilization of *Ceratitis capitata* has been the cobalt-60 gamma radiation.

However, it is known that radiations of a different nature may have different biological effects on insects (BANVILLE, 1964; CAUSSE et al., 1968).

Under this circunstance, we tried to evaluate the effect of the neutronic radiation on the mentioned species and simultaneously to compare its efficiency with that of cobalt-60 gamma radiation.

Neutrons are probably the oldest particles in the universe, aged about 10^{10} years, as predicted by RUTHERFORD in 1920. In 1930 these particles were produced by BOTHE e BECKER, but only in 1932 were they identified by CHADWICK (MAX BORN et al., 1969).

In 1936 ZIMMER (BURGER & BROERSE, 1974) used for the first time neutrons in entomology, when he irradiated *Drosophila melanogaster* Meigen, trying to produce cromossomic mutations, using for this purpose the reaction of litium Li (d,n) with an accelerator.

Although reports on the application of neutron radiation in insect sterilization are scarce, still we can quote relating to *Ceratitis capitata* those of CAUSSE et al., 1968; KLASSEN et al., 1969; PROVERBS, 1969; HOOPER, 1971; CAVALLORO & DELRIO, 1975.

Therefore one can say that the biological response of *Ceratitis capitata* submitted to neutronic radiation has not yet been fully studied and documented.

Neutrons from a nuclear reactor have a complex energy spectrum including neutrons of high energy which are the cause of high indirect ionization rates when they traverse living material.

According to KLASSEN et al., (1969) when a thermal neutron is captured by protoplasmic atomic elements it causes the emission of a gamma photon, which during it's subsequent history originates a succession of secondary ionizations.

Neutrons have a large capacity for transfering energy, or in other words have a high L.E.T. (Linear Energy Transfer) which varies in organic tissues from 8 to 45 keV/μm, in contrast with only 0.2 keV/μm in the case of gamma radiation (ZIRKLE, 1954).

Therefore when using neutronic radiation which has a high L.E.T., insects may be less damage, namely in what concerns the sexual competitiveness (because the relative biological effect is stronger). This is particularly important with *Ceratitis capitata* because an increase in the radiation doses weakens the competitiveness of the males (HOOPER e KATIYAR, 1971).

Materials and Methods

Irradiations with neutrons were carried out in the pool type Portuguese Research Reactor (RPI-Reactor Português de Investigação). The reactor core is composed of enriched uranium fuel elements located on the core grid and is emerged in water whose average temperature was about 28°C during the irradiations. The reactor can be operated up to a maximum power of 1 MW.

The RPI radiation is complex and is composed mainly by thermal neutrons, fast neutrons and gamma rays. The radiation flux is proportional to reactor power and decreses exponentially with the distance to the core in the different positions of irradiation at the core grid (Fig. 1).

For our irradiations the reactor power and the irradiation position were chosen such that the exposure time was neither too short nor too long. Due to

manual manipulation of the samples during the irradiation, the shortest the exposure time the larger the error in the integrated dose of radiation. With this type of manipulation a small variation in the irradiation time might change the dose received and thus alter the previously planned conditions which in turn can lead to serious alteration of the insect physiology.

Under this circunstances we agree with PROVERBS (1969) when he stated that neutron's dosimetry is particularly important in determining the radiation dose to be applied.

The physical conditions chosen for our irradiations were reactor power 0.5 kW and position 38 of core grid. For this conditions the radiation flux average values were as follows: thermal neutrons flux $0.8\times10^{9}n.cm^{-2}s^{-1}$; fast neutrons flux $0.7\times10^{8}n.cm^{-2}s^{-1}$; intensity of gamma radiation field 0.8×10^{2} Gy.h^{-1}.

The samples to irradiate were put inside a plastic bag which was then introduced inside a poliethylene cylinder 10 cm high and 4 cm diameter with screw-caps at both ends. The assembled set was then emerged in the pool water and introduced in the vertical tube located at position 38 of the core grid (Fig. 1). Five hundred pupae with 6 days of metamorphosical evolution were used in each experiment and exposed to two kinds of radiation: in the first study the biological material was submitted to total radiation composed of thermal and fast neutrons together with gamma radiation. In the second study only fast neutrons and gamma radiation were used. For this effect a 1 mm thick cadmium filter was used as absorber of thermal neutrons.

From the irradiated pupae the biological parameters analysed were the following: imago's emergence, detachment from puparium and normal adults. Sterility was also determined from the obtained fertility percentages of the crossings amongst normal and irradiated adult insects.

Results and Discussion

The sterilization of *Ceratitis capitata* with total neutronic radiation

Radiation doses of 24, 36, 40 and 48 Gy were applied to the pupae in this experiment.

The biological material was submitted to total neutronic radiation which consisted of 27.5% of thermal neutrons, 7.0% of fast neutrons and 65.5% of gamma radiation. The contribution of the considered radiations and the exposure times are shown in Fig. 2.

The imago's percentage of the irradiated pupae was comparable to that of the control group and their averages are very similar: 96.8% for the irradiated group and 96.5% for the control (Fig. 3).

Concerning the detachment from the puparium capacity, we observed once again a greater difficulty of the irradiated insects to free themselves from the involucre, when compared with those of the control group.

In the irradiated group the average percentages of the undetachment parameter were 1.2% for the males and 0.6% for the females, against only 0.4% and 0.2% for respectively males and females of the control group (Fig. 4).

It was clearly confirmed that in both the irradiated and control groups the males had higher indexes of undetachment from the puparium.

The average number of normal adults emerging from irradiated pupae were identical to those observed in the control group: 94% and 96% respectively (Fig. 5). For males, the obtained percentages were 45.4% and 46.4% respectively for the irradiated and control groups. For females the numbers were even more close, in fact almost equal (Fig. 5).

Concerning the effect of this radiation on the adult insect life span, we found that comparing with the control group and within the average duration of the experiment, 15 days, females lived for about the same time while the males had slightly shorter lives (Fig. 6).

According to CAUSSE et al., (1968) irradiation of pupae with total neutronic radiation with doses equal or higher than 10 Gy inhibits in the males the growth of the gonads leaving these insects with a very small residual fertility.

However, in our experiments, we found that complete sterility was obtained for females only with doses equal or higher than 24 Gy and higher than 40 Gy in the case of males. Thus females require lower radiation doses to induce sterility (Fig. 7).

The Sterilization of *Ceratitis capitata* with partial neutronic radiation

For this experiment the neutronic radiation was modified, using a 1 mm thick cadmium filter, which due to its high neutron absorption cross section is a powerful absorber of thermal neutrons.

Under this arrangement the neutronic radiation was restricted to a flux of fast neutrons (9.5%) and to gamma radiation (90.5%) (Fig.8).

In this experiment we used six doses of radiation: 18; 27; 31.5; 33; 34.5 and 39 Gy. Each dose has corresponded to given values of time and fluxes of fast neutrons and gamma radiation, as indicated in Fig. 8. The emergence rates of imagos emerging from irradiated pupae are very similar, not only between themselves, but also to those of the control group, as can be seen on the histogram in Fig 9. The average emergence percentage was 94.7% for irradiated pupae and 96.8% for the control group.

According to HOOPER (1971) doses of 19 Gy have no noxious effect in the emergence percentage. However, even for much higher doses such as those used in our study (39 Gy) the average emergence percentage of adults was always quite high and around 95% (Fig. 9).

Regarding the parameter for the undetachment from puparium the results obtained were very similar to those of the previous experiment with the higher percentage corresponding to the irradiated group.

Once again we observed a greater difficulty of detachment by the males (Fig 10). The obtained percentage of normal adults, after irradiating the pupae with this kind of radiation, having only fast neutrons and gamma rays, was about

93% and thus very much the same as in the previous essay which had a percentage of 94% (Fig. 11).

As for the survival rate of adults within the average duration of the experiment it was very similar to that of the control group as can be confirmed by the corresponding histogram (Fig. 12).

Although HOOPER (1971) mentioned that fast neutrons can sterilize females with doses between 7 and 11 Gy, in our experiments, where we applied doses under a careful dosimetry conditions, infertility was only reached with doses over 18 Gy (Fig. 13). However it must be mentioned that with total radiation of neutrons, females became infertile only with doses of 24 Gy or more.

As far as males are concerned, HOOPER (1971) mentions that with a dose of 19 Gy, he obtained sterilization rates of about 98% without shortening of the longevity.

CAVALLORO and DELRIO (1974) mentioned that with this dose of radiation of fast neutrons, a percentage of 93% of sterile males can be reached.

In this experiment we observed that males, being less sensitive to radiation than females, became completely sterile when submitted to doses of 33 Gy or more. There is a difference between this and the previous estimate where 40 Gy of total radiation were necessary to obtain a complete sterilization of the males.

In our study we observed obvious differences in sterilization results between total and partial neutronic radiation. We may conclude that the latter, formed only by fast neutrons and gamma radiation, leads to smaller doses necessary to completely sterilize adults, when compared to the doses of complete radiation used for the same purpose.

Conclusion

Neutrons have a much higher biological relative efficiency than gamma radiation.

As mentioned by CAUSSE (1968), CAVALLORO and DELRIO (1974) and confirmed by us, the doses of neutronic radiation applied to obtain the same degree of adult sterility are smaller than those of only gamma radiation necessary to reach the same results. This is true because neutronic radiation induces more dominant letal mutations (HOOPER, 1971). Under this circunstance the same sterilization effects can be obtained with radiation doses 2 to 3 times smaller, which results in a decrease of the adverse effects in the physiology and behaviour of the insect.

In this case, we can conclude that neutrons are effective in the med fly *Ceratitis capitata* sterilization, causing no adverse effects in the biological parameters analysed, namely in the emergence rate of imagos, in the detachment from puparium capacity and in the normal adults percentage.

As for competition CAVALLORO and DELRIO (1974) stated that males sterilized with fast nestrons had a sexual appetence four times higher than those sterilized with gamma radiation.

However we accept this statement with some reserve. In fact, in all our experiments the males exposed to that radiation effects revealed some decrease in

their intrinsic characteristics, namely in it's flying capacity and sexual appetence, thus becoming less competitive. Although we believe that this kind of radiation has great potential in the sterile male technique field.

Further theoric and pratical studies must still be carried out before the desirable results can be obtained.

Acknowledgement

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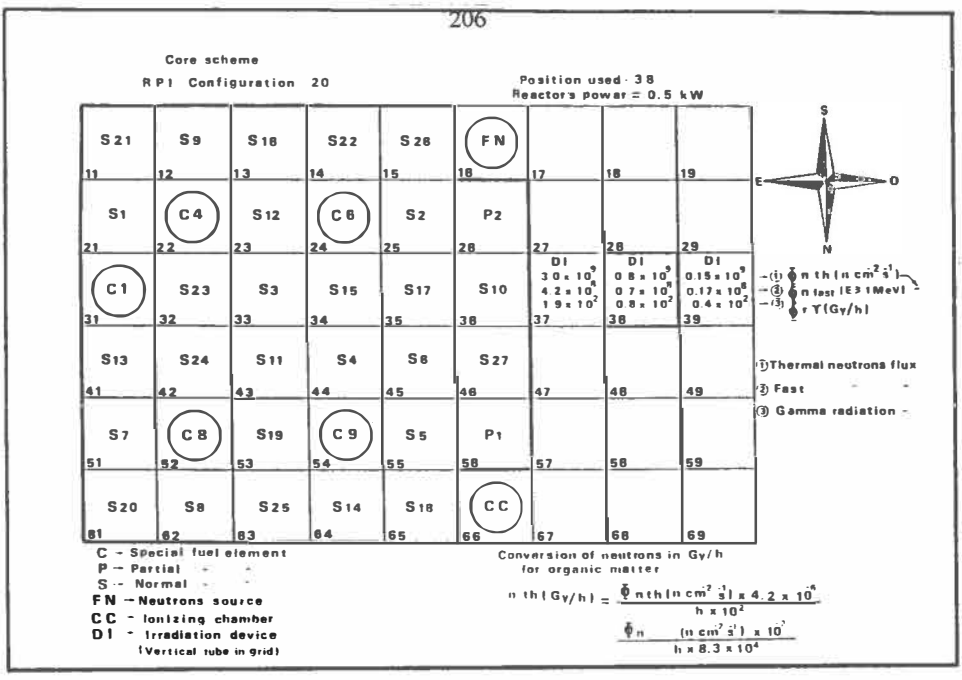


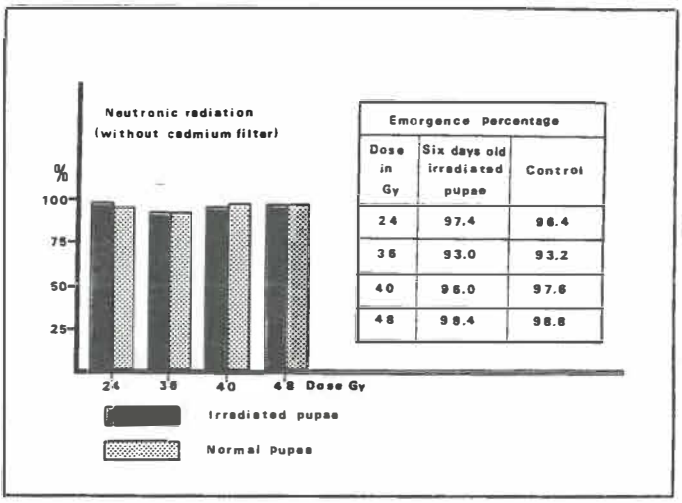
Fig. 1 - RPI's core scheme, used for sterilization of *Ceratitis capitata* at 0,5 kW power.

Neutronic radiation				
Physical characteristics on irradiation position				
Irradiation position	Power (kW)	Fluxes		
		Φ n th	Φ n fast	Φ rad T
		0.8 x 10^9	0.7 x 10^8	0.8 x 10^2
38	0.5	n cm^-2 s^-1		Gy/h

Contribution of radiation in relation to different times and doses							
Time (min)	Dose (Gy)	Without cadmium filter					
		Φ n th		Φ n fast		Φ rad T	
		%	Gy	%	Gy	%	Gy
11.79	24	27.5	6.60	7.0	1.68	65.5	15.72
17.70	36	27.5	9.90	7.0	2.52	65.5	23.58
19.66	40	27.5	11.00	7.0	2.80	65.5	26.20
23.60	48	27.5	13.20	7.0	3.36	65.5	31.44

Φ n th — Thermal neutrons flux
Φ n fast — Fast -
Φ rad T — Gamma radiation -

Fig. 2 - Contribution of the various radiations for different times and doses in irradiation position 38 at reactor power of 0,5 kW (without cadmium filter).

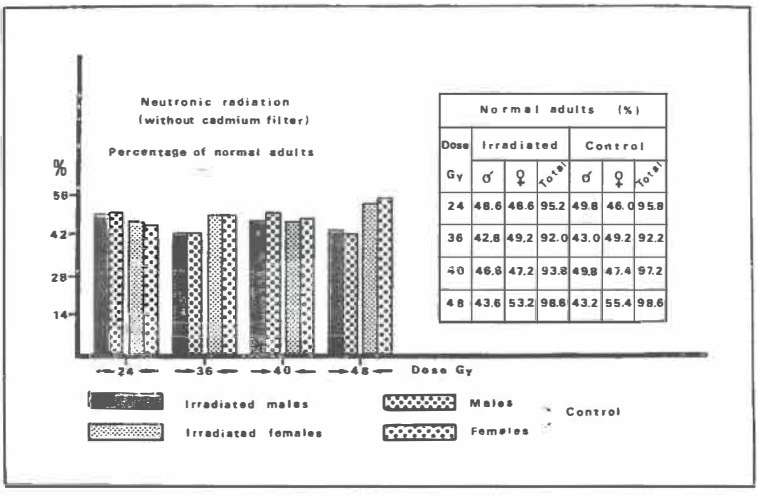


Emergence Percentage		
Dose in Gy	Six days old irradiated pupae	Control
24	97.4	98.4
36	93.0	93.2
40	96.0	97.6
48	98.4	98.8

Fig. 3 - Emergence percentage of adult insects from pupae irradiated with total neutronic radiation.

Neutronic radiation (without cadmium filter)						
Undetachment from puparium (%)						
Dose in Gy	Six days old irradiated pupae			Control		
	Males	Females	Total	Males	Females	Total
24	1.6	0.6	2.2	0.6	0.0	0.6
36	0.8	0.2	1.0	0.6	0.4	1.0
40	1.6	0.6	2.2	0.4	0.0	0.4
48	0.8	0.8	1.6	0.0	0.2	0.2

Fig. 4 - Undetachment from puparium by adult insects emerging from pupae irradiated at different neutronic radiation doses.



Normal adults (%)						
Dose	Irradiated			Control		
Gy	♂	♀	Total	♂	♀	Total
24	48.6	46.6	95.2	49.8	46.0	95.8
36	42.8	49.2	92.0	43.0	49.2	92.2
40	46.6	47.2	93.8	49.8	47.4	97.2
48	43.6	53.2	98.6	43.2	55.4	98.6

Fig. 5 - Percentage of normal adult insects, emerging from pupae irradiated with total neutronic radiation.

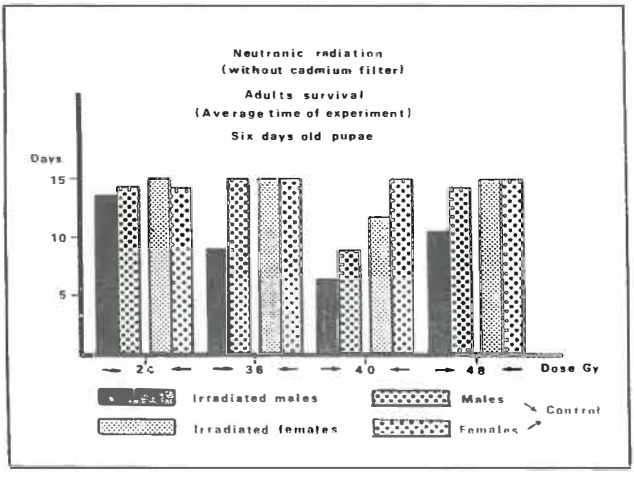


Fig. 6 - Average survival time of adult insects emerging from pupae irradiated with total neutronic radiation.

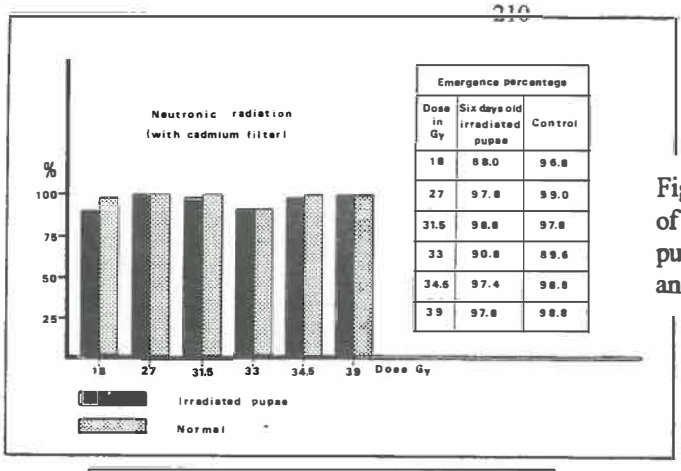
Neutronic radiation				
Without cadmium filter				
Dose in Gy	Total fertile eggs / female			
	Adults matings — Six days old pupae			
	♀I x ♂I	♀I x ♂N	♀N x ♂I	♀N x ♂N
24	0	0	5	82
36	0	0	4	98
40	0	0	0	110
48	0	0	0	92

Fig. 7 - Fecundity comparative results of different matings amongst normal and irradiated adult insects.

Neutronic radiation							
Physical characteristics in irradiation position							
Irradiation position	Power (kW)	Fluxes					
		$\bar{\Phi}$ n th		$\bar{\Phi}$ n fast		$\bar{\Phi}$ rad γ	
		0.8×10^{9}		0.7×10^{8}		0.8×10^{2}	
38	0.5	n cm^{-2} s^{-1}				Gy/h	
Contribution of radiation in relation to different times and doses							
Time (min)	Dose (Gy)	With cadmium filter - 1 mm					
		$\bar{\Phi}$ n th		$\bar{\Phi}$ n fast		$\bar{\Phi}$ rad γ	
		%	Gy	%	Gy	%	Gy
12.21	18.0	—	—	9.5	1.71	90.5	16.29
18.31	27.0	—	—	9.5	2.56	90.5	24.44
21.27	31.5	—	—	9.5	2.99	90.5	28.51
22.38	33.0	—	—	9.5	3.14	90.5	29.86
23.40	34.5	—	—	9.5	3.28	90.5	31.22
26.46	39.0	—	—	9.5	3.71	90.5	38.29

$\bar{\Phi}$ n th — Thermal neutrons flux
$\bar{\Phi}$ n rap — Fast " "
$\bar{\Phi}$ rad γ — Gamma radiation flux

Fig. 8 - Contribution of the various radiations for different times and doses in irradiation position 38 at reactor power of 0,5 kW (with cadmium filter).

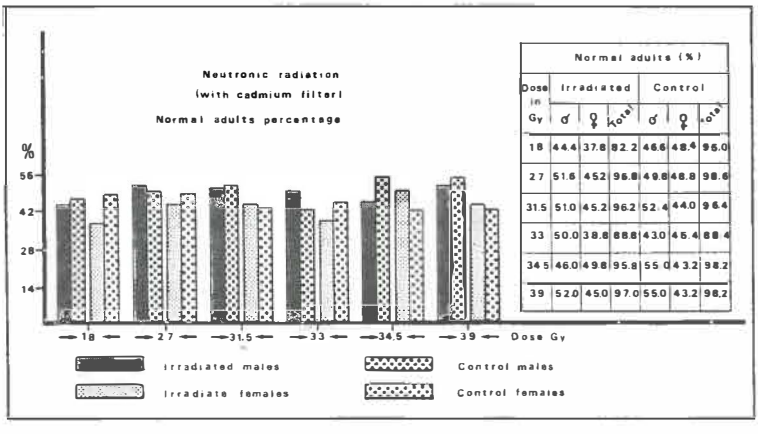


Emergence percentage		
Dose in Gy	Six days old irradiated pupae	Control
18	88.0	96.8
27	97.8	99.0
31.5	98.8	97.8
33	90.8	89.6
34.5	97.4	98.8
39	97.8	98.8

Fig. 9 - Emergence percentage of adult insects emerging from pupae irradiated with fast neutrons and gamma radiation only.

Neutronic radiation (with cadmium filter)						
Undetachment from puparium (%)						
Dose in Gy	Six days old irradiated pupae			Control		
	Males	Females	Total	Males	Females	Total
18	4.2	1.6	5.8	1.0	0.8	1.8
27	0.6	0.4	1.0	0.4	0.0	0.4
31.5	0.4	0.2	0.6	1.2	0.2	1.4
33	1.6	0.4	2.8	0.4	0.8	1.2
34.6	1.2	0.4	1.8	0.4	0.2	0.6
39	0 8	0.0	0.8	0.4	0.2	0.6

Fig. 10 - Undetachment from puparium by adult insects emerging from pupae irradiated at different neutronic radiaton doses.



Normal adults (%)						
Dose in Gy	Irradiated			Control		
	♂	♀	total	♂	♀	total
18	44.4	37.8	82.2	46.6	48.4	95.0
27	51.6	45.2	96.8	49.8	48.8	98.6
31.5	51.0	45.2	96.2	52.4	44.0	96.4
33	50.0	38.8	88.8	43.0	45.4	88.4
34.5	46.0	49.8	95.8	55.0	43.2	98.2
39	52.0	45.0	97.0	55.0	43.2	98.2

Fig. 11 - Percentage of normal adult insects, emerging from irradiated pupae with fast neutrons and gamma radiation only.

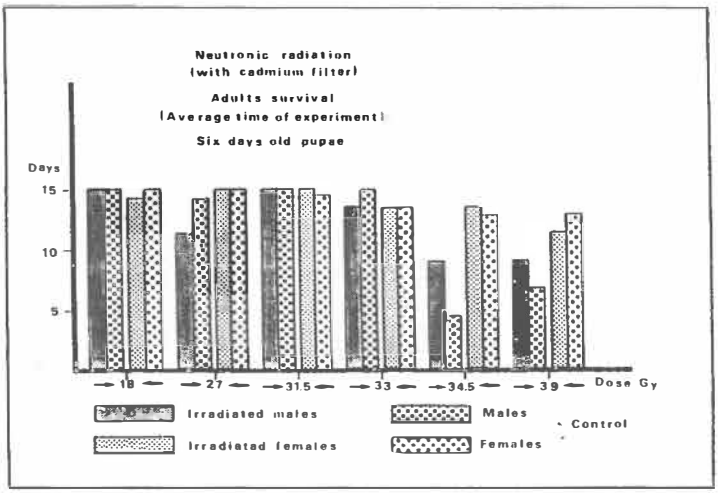


Fig. 12 - Average survival time of adult insects emerging from pupae irradiated with fast neutrons and gamma radiation only.

Neutronic radiation				
With cadmium filter				
Dose in Gy	Total fertile eggs / female			
	Adults matings - six days old pupae			
	♀I x ♂I	♀I x ♂N	♀N x ♂I	♀N x ♂N
18	0	0	2	155
27	0	0	2	99
31.5	0	0	6	129
33	0	0	0	140
34.5	0	0	0	128
39	0	0	0	128

Fig. 13 - Fecundity comparative results of different matings amongst normal and irradiated adult insects.

USE OF STERILE MALE-ONLY RELEASES AS "BIOLOGICAL PESTICIDES" FOR CONTROL OF MEDITERRANEAN FRUIT FLIES IN COMMERCIAL FRUIT PRODUCTION.

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Summary

The Sterile Insect Technique (SIT), has been widely applied to exclude or eradicate the Mediterranean fruit fly (medfly). In large scale (SIT) programmes, standard strains (ie. having both sexes) are reared and released. However, the damage that sterile female stings produce has hampered the introduction of the SIT as a control measure in commercial fruit producing areas.
The Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture has been sponsoring and carrying out research to develop medfly sexing strains. Recently, as a result of this effort, the IAEA laboratories located at Seibersdorf, Austria, have developed genetic sexing strains based on a temperature sensitive lethal (tsl) mutation. This so-called "second generation" of genetic sexing (GS) strains allows female discrimination at an early (embryonal) stage, while the "first generation" of GS (ie. pupae colour dimorphic strains) requires later separation (ie. no reduction in costs of larval rearing and others). In addition a tsl-based strains has been obtain that is essentially stable under mass rearing conditions. This represents a major breakthrough because both of these attributes are considered indispensable for genetic sexing strains with any potential to replace standard strains in large scale sterile medfly production facilities.
In addition to the significant savings in insect release and field monitoring, field testing of genetic sexing strains has shown several-fold increases in the effectiveness of the SIT as compared with standard strains. When releasing both males and females, sterile males are apparently not used effectively, because a) they use their limited sperm largely to mate with sterile females and b) because they do not disperse in the presence of these females. When releasing only sterile males, however, they disperse more effectively in search of wild females and compete more intensely with wild males for wild female matings.
As a result of the availability of usable male-only strains, and the demonstration of their increased effectiveness, the applicability of the SIT against medfly has increased. Highly developed commercial fruit growing regions, that previously had excluded application of SIT because of the fruit damage, are now reconsidering control or free area/exclusion programmes using sterile males.

1-Introduction

The Mediterranean fruit fly (medfly) is among the most important insect pests in the world. Its wide range covers over 200 types of fruit and vegetables (Liquido et al. 1991), of which at least one half are of commercial value. Control of this pest is a top priority for fruit producers and exporters. Commercial interaction is hampered by

the placement of quarantine measures to avoid movement of this pest, with the subsequent damage to the economies of fruit producing countries.

Several chemical approaches have been developed to overcome/reduce their incidence. However, these chemical pre-and post-harvest control practices are being reclassified as harmful to both humans and environment, causing paralysis of fresh fruit control and trade. In addition, environmental concerns require the introduction of more friendly practices.

Research on the use of the Sterile Insect Technique (SIT) against fruit flies, including the Mediterranean fruit fly, began in the mid-1950's and since then, this technique has developed enormoulsly, culminating in a number of important successes in controlling or eradicating fruit fly pests, particularly in the Western Hemisphere, Japan and Australia. Klassen et al. (1993), in a historical review, list all previous and ongoing SIT field applications against fruit flies, ranging from research field trials, applied suppression/control activities to massive exclusion/eradication programmes.

Numerous technological developments at research laboratories throughout the world have contributed to increase the sterile insect production capacity (Vargas 1989) and the subsequent application of the SIT. The large number of already operating tephritid mass rearing facilities in the world, and in particular the number of recently built or planned facilities (Table I) is in itself an indication of the progress made in the developement of this technology. It reflects also the interest of producers and governments in seeking an enviromentally sound approach that allows to overcome non-tariff barrier to international trade of fresh fruit.

Classical biological control alone, has proven not to be effective to protect fruit production from fruit fly damage due to the low tolerance threshhold of commercial fruit production. A number of fresh fruit importing countries have an absolute zero tolerance. Producers are therefore forced to rely on weekly insecticide-bait sprays to protect commercial fruit from fruit flies (Rössler 1989). Such sprays offset otherwise viable integrated pest management (IPM) schemes, often including augmentative releases of natural enemies, that are in place against a number of other fruit pests (Ehler & Endicott 1984). Existing citrus IPM programmes are in particular very promising in the Mediterranean region, however, their implementation depends upon a non-chemical control of the Mediterranean fruit fly (FAO 1992).

As a result of the continuous use of insecticide treatments against the medfly, growers presently face increasing environmental problems related not only to public perception of pesticide residues in orchards and their products, but particularly to increasing secondary pest outbreaks (Troetschler 1983; Hoy & Dahlsten 1984; Daane et al. 1990; Hoelmer & Dahlsten 1993). One example is Israel, where for the last thirty years large scale, centrally organized aerial bait spray applications have been carried out to be able to export medfly free citrus and other fresh fruit to Europe (Cohen & Cohen 1967), and where scale insects and other secondary pest problems have become a common occurrence (Cohen et al. 1987). More environment-friendly and target specific techniques, such as the SIT as well as more sophisticated genetic control methods and/or augmentative releases of natural enemies, are therefore receiving renewed attention.

Table I. Comparison of Fruit Fly Rearing Facilities in Europe and the Rest of the World

EUROPE *	
FLY SPECIES	LOCATION
C. capitata	Seibersdorf, Vienna, Austria
C. capitata	Heraklion, Crete, Greece
B. olea	Demokritos, Athens, Greece
ASIA **	
FLY SPECIES	LOCATION
C. capitata	Perth, W. Australia, Australia
B. cucurbitae	Naha, Okinawa, Japan
B. tryoni	Perth, W.A., Australia
B. dorsalis	3 locations around Bangkok, Thailand
B. dorsalis	Quezon City, Luzon, Philippines
AMERICA **	
FLY SPECIES	LOCATION
C. capitata	Tapachula, Chiapas, Mexico
C. capitata	Waimanalo, Hawaii, USA
C. capitata	Petapa, Guatemala, Guatemala
C. capitata	La Molina, Lima, Peru
C. capitata	Mendoza, Mendoza, Argentina
C. capitata	Honolulu, Hawaii, USA
C. capitata	Arica, Region I, Chile
C. capitata	San José, Univ.C.R., Costa Rica
C. capitata	San Juan, San Juan, Argentina
A. ludens	Tapachula, Chiapas, Mexico
A. ludens	Mission, Texas, USA
A. obliqua	Tapachula, Chiapas, Mexico
A. suspensa	Gainsville, Florida, USA

* All European facilities are small scale, producing in the range of 1 - 10 million flies per week.

** Most Non-European facilities are of large scale, some producing up to 600-700 million flies per week.

2-Development of genetic sexing strains.

One of the obstacles hindering a faster and wider adoption of the Sterile Insect Technique (SIT) against the medfly, particularly in commercial citrus producing regions, is the damage fruit suffers due to sterile female stings. These females, although not able to lay eggs, still have the drive to bore into fruit thereby injuring it. In addition, due to the boring as well as the phenomenon of regurgitation (Cayol et al. 1993), females of medfly have been shown to be active vectors of fruit rot fungi as well as phytopathogenic bacteria.

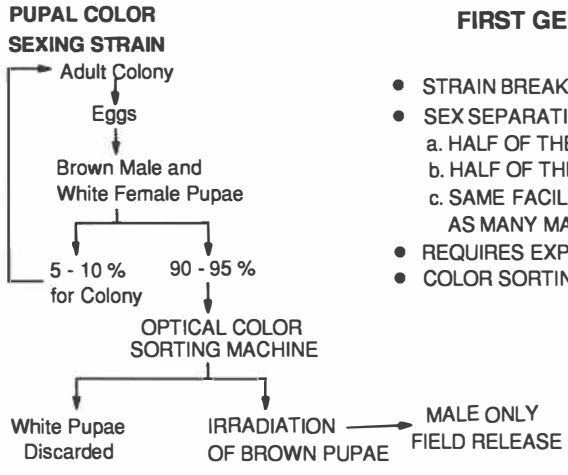
To overcome this obstacle, the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture has played a leading role sponsoring and carrying out research to develop medfly genetic sexing strains. These are strains in which large scale separation of sexes is possible through genetic manipulation, allowing male-only sterile fly releases.

The so-called "first generation" genetic sexing strains, based on pupal color mutations, were developed at various laboratories (Rössler 1979a, 1979b; Robinson & van Heemert 1982; Bush-Petersen et al. 1988). Although utilized for various experimental pilot field tests, these strains never reached the stage of practical application in ongoing field programmes. Rapid strain breakdown due to recombination, late separation of sexes at the pupal stage (resulting in wasted female pupae and larval diet), and the need for expensive sex sorting machines were the main reasons for this lack of utilization.

Recently, genetic sexing strains based on a temperature sensitive lethal (*tsl*) mutation have been developed (Franz et al. 1993). Unlike previous pupal color sexing strains, these "second generation" sexing strains allow female killing at an early (embryonal) stage due to thermal treatments. Eggs, harvested from the adult colony are subjected to a bubbling in an inexpensive hot water bath instead of the normal bubbling at ambient temperature. In addition, as a result of the development of translocations with chromosome breakpoints close to the sexing genes, they are essentially stable under mass rearing conditions. This represents a major breakthrough because a) inexpensive female elimination and b) strain stability were considered indispensable attributes for genetic sexing strains with any potential to replace standard strains.

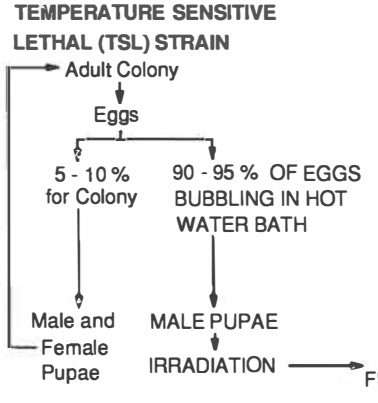
The mass rearing system for the most promising tsl sexing strain, called Vienna-42, has been developed (Hendrichs et al. unpublished data). Furthermore, the behavior of this tsl sexing strain has been tested in extensive greenhouse and field cage studies (Hendrichs et al. unpublished data), as well as a survival-dispersal study in the field (Hendrichs et al. 1993). These tests showed adequate behavior and mating competitiveness. As a result, the medfly mass rearing facility in Guatemala is presently converting one of its two modules to initiate mass rearing of a Vienna-42 strain in which the genetic background is of Guatemalan origin (ie. hybrid strain). Adaptations needed in existing rearing facilities to mass rear tsl sexing strains are relatively minor, particularly in those already using the "larval popping" system. Rearing this strain requires adequate temperature control systems, especially in the larval rearing area producing males and females for colony maintenance. In addition, egg production has to be increased approximately three-fold if the male production of a given facility is to be doubled.

FIG. 1. GENETIC SEXING IN MEDFLY MASS REARING SYSTEMS



FIRST GENERATION GENETIC SEXING

- STRAIN BREAKDOWN RAPID
- SEX SEPARATION IS LATE, THEREFORE:
  a. HALF OF THE FINAL PRODUCT IS DISCARDED
  b. HALF OF THE LARVAL DIET IS WASTED
  c. SAME FACILITY CANNOT PRODUCE TWICE AS MANY MALES
- REQUIRES EXPENSIVE SORTING MACHINE
- COLOR SORTING ACCURACY ONLY 95-98%



SECOND GENERATION GENETIC SEXING

- STRAIN BREAKDOWN IS VERY SLOW
- FEMALES ELIMINATED AT EARLY STAGE:
  a. NO FINAL PRODUCT IS DISCARDED
  b. NO LARVAL DIET IS WASTED
- NO EXPENSIVE SORTING EQUIPMENT NECESSARY
- ACCURACY OF SEX SEPARATION IS 99.9%
- SAME FACILITY CAN PRODUCE NEARLY TWICE AS MANY STERILE MALES
- FIELD MONITORING IS FACILITATED BECAUSE OF ABSENCE OF STERILE FEMALES

3-Effectiveness of Male-Only Strains

The importance of sterile females in relation to their effect on the SIT has been debated for some time. There has been the theoretical argument that sterile females are an important "sponge" of wild male sperm (Kaneshiro, personal communication). The opposite argument is that wild males have a relatively unlimited amount of sperm as indicated by fruit fly male-annihilation programmes in which over 99% of males have to be eliminated in order to achieve a reduction in population. On the other hand it has been found that under conditions of limited fruit availability, sterile females are territorial on fruit, interfering with the oviposition of wild females (McInnis & Wong 1990).

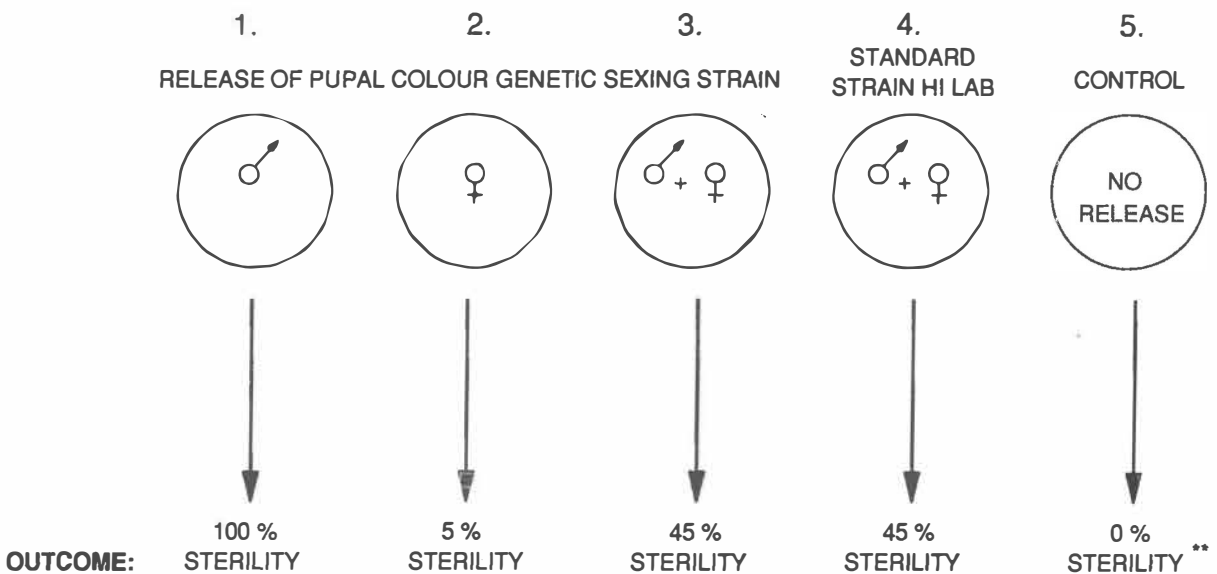
Even though pupal color strains have only been the first step in the direction of developing viable medfly sexing strains, they have been extremely useful to test the impact of sterile insect releases with a biased sex ratio. Results of several field cage mating tests (Robinson et al. 1986; McInnis et al. 1986; Hendrichs et al. unpublished data) have indicated a significant advantage in terms of mating and induced sterility for male-only releases compared to male and female releases.

Results of a recent field pilot test in Hawaii (McInnis et al. 1993), lasting over half a year, and involving 5 separate field sites, have confirmed that i) female-only releases have essentially no impact on sterility, ii) there is a several-fold increase in the effectiveness of the SIT when male-only releases are made compared to male and female releases (Fig. 2). Under normal SIT conditions the sex ratio remains 1:1, and mating between sterile insects often starts in the release cages or bags before release and continues at the release site. In male-only releases, however, this cannot happen and sterile males save their limited sperm and are forced to compete more intensively for the small number of wild females (in proportion to large numbers of males). Finally, sterile males appear to disperse more, as shown by increased capture ratios, probably because they have to search for wild females. This effect has been found in the Hawaiian field test (McInnis et al. 1993), in Israel (Nitzan et al. 1993) and release-recapture studies in Guatemala (Bloem et al. unpublished data) and Greece (Hendrichs et al. 1993).

4-Male-Only Strains as Biopesticides.

Another pioneering field pilot test, also using a medfly pupal color sexing strain (Nitzan et al. 1993), has shown that male-only releases are a viable alternative to conventional chemical control. This two-years test, partially financed by IAEA, was carried out in two isolated ca. 500 ha fruit producing areas (kibutz) in Israel. In the orchards of the control kibutz routine aerial bait-spray was applied at 8 to 10 day intervals, whereas in the other kibutz sterile males of the pupal colour sexing strain, produced, irradiated and shipped from Vienna, were released by ground or air (Fig. 3). Results collected over two seasons, involving inspection of many thousand fruits, indicated that there was no difference in fruit infestation between the two treatments, confirming that male-only SIT is indeed a viable and much more environment-friendly alternative to conventional insecticide treatment.

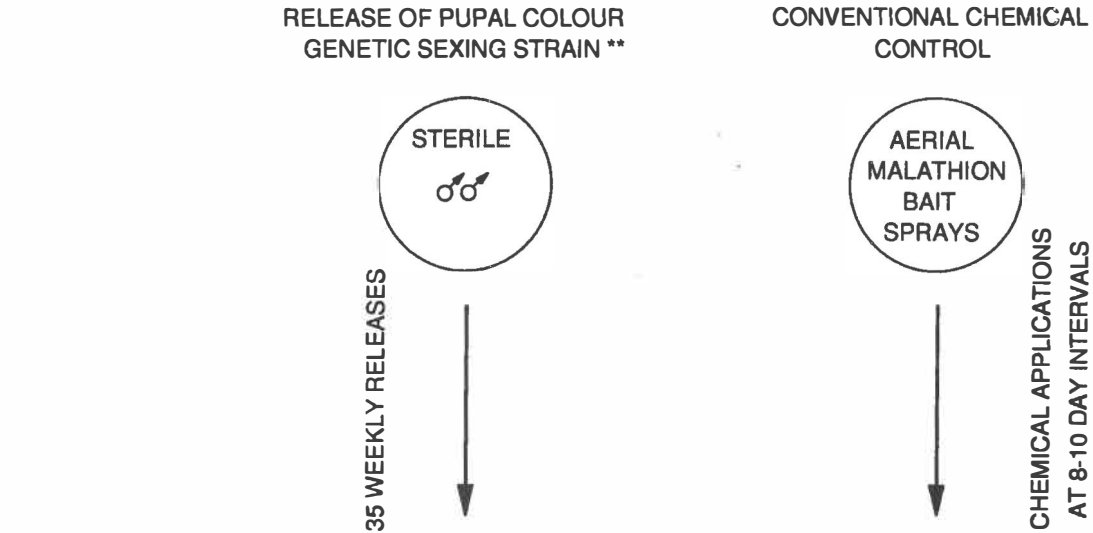
FIG. 2. INDUCED STERILITY IN MEDFLY POPULATIONS BY RELEASE OF DIFFERENT SEX RATIOS OF STERILIZED FLIES OF STANDARD OR PUPAL COLOR GENETIC SEXING STRAIN IN FIVE TEST AREAS *



* 6 month experiment over 5 isolated coffee producing areas in Kauai, Hawaii (data from McInnis et al. 1993).

** Sterility results corrected based on natural sterility in the control

FIG. 3. CONTROL OF MEDFLY POPULATIONS BY RELEASE OF STERILIZED MALES FROM A PUPAL COLOR GENETIC SEXING STRAIN COMPARED TO CONVENTIONAL CHEMICAL CONTROL IN TWO ISOLATED TEST AREAS *



* Two 500-hectare fruit production farms (kibutz) in Israel (data from Nitzan et al. 1993).
** Two bait spray applications prior to beginning of sterile male releases.

5-Conclusions

The advantages of using GS strains in fruit fly SIT programmes are overwhelming (Table II). The initial reasons for pursuing a programme on medfly genetic sexing were two-fold: a) eliminate the damage caused by sterile stings and b) reduce the overall cost of sterile fly production and release. Another expected benefit was the increased safety of this technology making the accidental release of non-sterile females impossible.

Testing some of these sexing strains in the field, however, has shown that increased overall effectiveness of the SIT is probably as/or even more important. Sterile males, in the absence of sterile females, do not remain near the release site where they mate mainly with sterile females, but spread in search of wild females and compete more effectively with wild males.

Another important benefit is the potential to simplify field monitoring and detection. Reducing the cost of these activities, which represent at least a third of the cost of the SIT, would also decrease significantly the overall cost of fruit fly SIT programmes. Using exclusively fruit sampling and female attractant-baited traps in combination with male-only sterile fly releases would focus exclusively on detecting wild females and their offspring, thereby eliminating all present uncertainties and labor related to discriminating between thousands of recaptured sterile and wild flies.

A further important benefit of using a GS strain is the increased quality of the released male (D. Orozco, personal communication). At present, medfly pupae have to be irradiated 48 hrs before emergence to avoid any risk of some females advancing in their oogenesis if irradiated at a later time (Williamson et al. 1984). However, irradiation of only male pupae can occur only 24 hours before emergence. The result is increased quality of the sterile males.

Finally, in the absence of sterile females in the released material, the potential applicability of the SIT against tropical/subtropical fruit flies increases considerably. It will no longer be a technique to be applied solely by governments at an area-wide level for special situations requiring eradication or exclusion. Rather, the potential exists for using sterile males as a "biological insecticide" during fruiting seasons to replace insecticide-bait sprays. These can even be used in combination with augmentative releases of parasitoids (Knipling 1992; Jimenez & Castillo 1992). Integrated pest management systems, presently only effective for early maturing varieties, could be applied in all commercial orchards with the availability of such a non-disruptive "biological control" of medfly. In many areas of the Mediterranean region, where few flies emerge after the winter in spring or early summer, the possibility also exists to overflood these few survivors by early area-wide releases of sterile males in order to suppress breeding populations. Thereby the large build-up of wild populations that would require chemical control during the fall fruiting season is delayed. Either of these approaches may be a more appropriate strategy than eradication in many geographic or socioeconomic situations (Bateman 1989). Furthermore, under such situations, these suppression approaches using SIT are more cost effective than SIT for eradication (Mumford et al. 1993). Finally, conditions for the commercialization of sterile fly production for such sustainable SIT application may be more favorable under these suppression scenarios.

Table II. ADVANTAGES OF USING GENETIC SEXING STRAINS IN FRUIT FLY SIT PROGRAMMES

- No Damage to Fruit

a) absence of sterile females results in fruit with no oviposition stings

b) absence of sterile females results in a drastic reduction of active vectors of fruit rot fungi and phytopathogenic bacteria

- Considerable Savings

a) in rearing

b) irradiation, packing and transport

c) in release

- Increased Safety

a) accidental release of non-irradiated batches would include only males

b) escaping females from mass rearing facility would have reduced fitness

- Several-Fold Increase in Effectiveness of SIT

a) limited sperm of sterile males is not wasted in matings with sterile females

b) sterile males disperse much more in absence of sterile females

c) sterile males compete much more for wild females

d) sterile males can be released at a more mature age reducing thereby losses to predation before males are sexually most effective

- Simplified and more precise field monitoring activities when using female attractants

a) there is no need to process thousands of recaptured sterile male flies

b) there is no uncertainty due to lack of color marking or inconclusive dissection

- Increased Male Quality

a) male pupae can be irradiated 24 hrs before emergence, instead of the 48 hrs presently required when also irradiating female pupae

- Increased Applicability of SIT

a) to commercial fruit growing regions where eradication attempts had been excluded because of oviposition damage to fruit

b) as a biological insecticide to replace chemical bait-sprays during fruiting seasons

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STERILITY AND MATING COMPETITIVENESS OF MALE *CERATITIS CAPITATA* AS AFFECTED BY GAMMA RADIATION AND DIMETHOATE INSECTICIDE

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Summary

In the present work, the effects of pupal gamma-irradiation (50, 70, 90 Gy) or adult treatment with dimethoate insecticide (0.93 ppm) on the male sterility and mating competitiveness of the medfly, ***Ceratitis capitata*** (Wied.) were studied.

Results indicated that treatment of the adult medfly with dimethoate had no clear effect on male fertility whether this male mated with treated or normal females. However, gamma irradiation decreased drastically hatchability of eggs laid by normal or treated (dimethoate) females, when mated with irradiated males at all gamma doses.

This reduction in egg hatchability increased with increasing the gamma dose. Irradiated females did not lay eggs at any of the gamma doses tested. At all mating combinations, the male mating competitiveness was decreased especially when irradiated males were confined with treated males for mating with normal or treated females. The highest male mating competitiveness values were recorded when irradiated or irradiated treated male were confined with normal males for mating with treated females (0.89 and 0.90, respectively). However, the lowest values were observed when irradiated or irradiated treated males were confined with treated males for mating with normal females (0.20 and 0.49, respectively).

1 – Introduction

An important part of research on the development of sterile insect technique (SIT) should involve investigations on ways to suppress high natural populations before

contemplating the use of this method. A high level of suppression by chemical means may be possible in a well coordinated program. In Egypt, Dimethoate was and still used for the medfly chemical control.

A series of laboratory experiments were performed on the medfly to study the expected effects of using SIT programme after population suppression with Dimethoate on the released flies.

2-Materials and Methods

Medflies were reared in the laboratory at 25±2°C on the usual local synthetic medium. Pupal irradiation was done using a Co-60 gamma cell unit with a dose rate of about 2.4 Gray/minute during the experimental period. Dimethoate (40%) was used as an adulticide in a concentration of 0.93 ppm applied as a dry film sprayed on the inner surface of the jars. Newly (1-24 h) emerged adults were introduced to the jars and kept for 24 hours at 25±2°C. The still living adults were then used for the present experiments. Combined effect on male mating competitiveness was achieved by irradiation of full grown pupae with 90 Gy then the emerged adults were exposed to dimethoate for 24 hours. Mating competitiveness values as well as the expected egg hatch were calculated according to Fried (1971) for the ratio 3:1:1 (irradiated, I or irradiated treated, IT males: normal, N or treated, T males: normal or treated females.

3-Results

Fig. (1) shows the effect of treating males with dimethoate (0.93 ppm) on the egg hatchability when mated with females treated (dimethoate) as adults or irradiated in the pupal stage with 50, 70 or 90 Gy). It is clear from the figure that hatchability of eggs laid by normal females mated with treated males was not affected. Moreover, there was no significant difference in the percentage of egg hatchability when treated males were mated with treated females. The data show also that when irradiated or irradiated treated females were inseminated with normal or treated males no egg laying was observed.

Fig. (2) illustrates egg hatchability of normal, treated and irradiated treated females when mated with irradiated males. When irradiated males mated with normal females the hatchability of the laid eggs significantly decreased at all

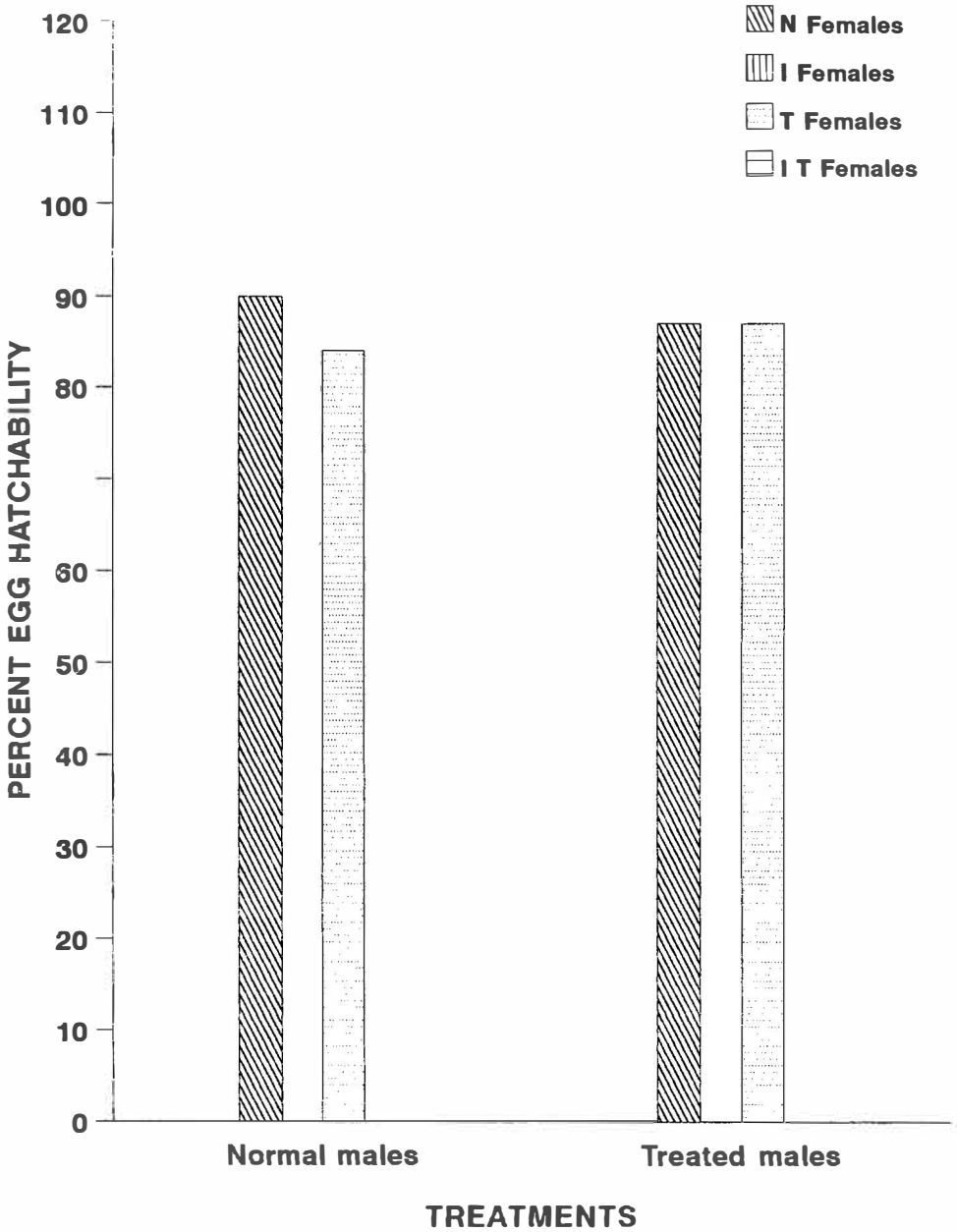

FIG. (1): Effect of treating adult males of Ceratitis capitata with Dimethoate (0.93 ppm) on egg hatchability when mated with normal, treated (Dimethoate), gamma irradiated (as pupae) or irradiated treated females.

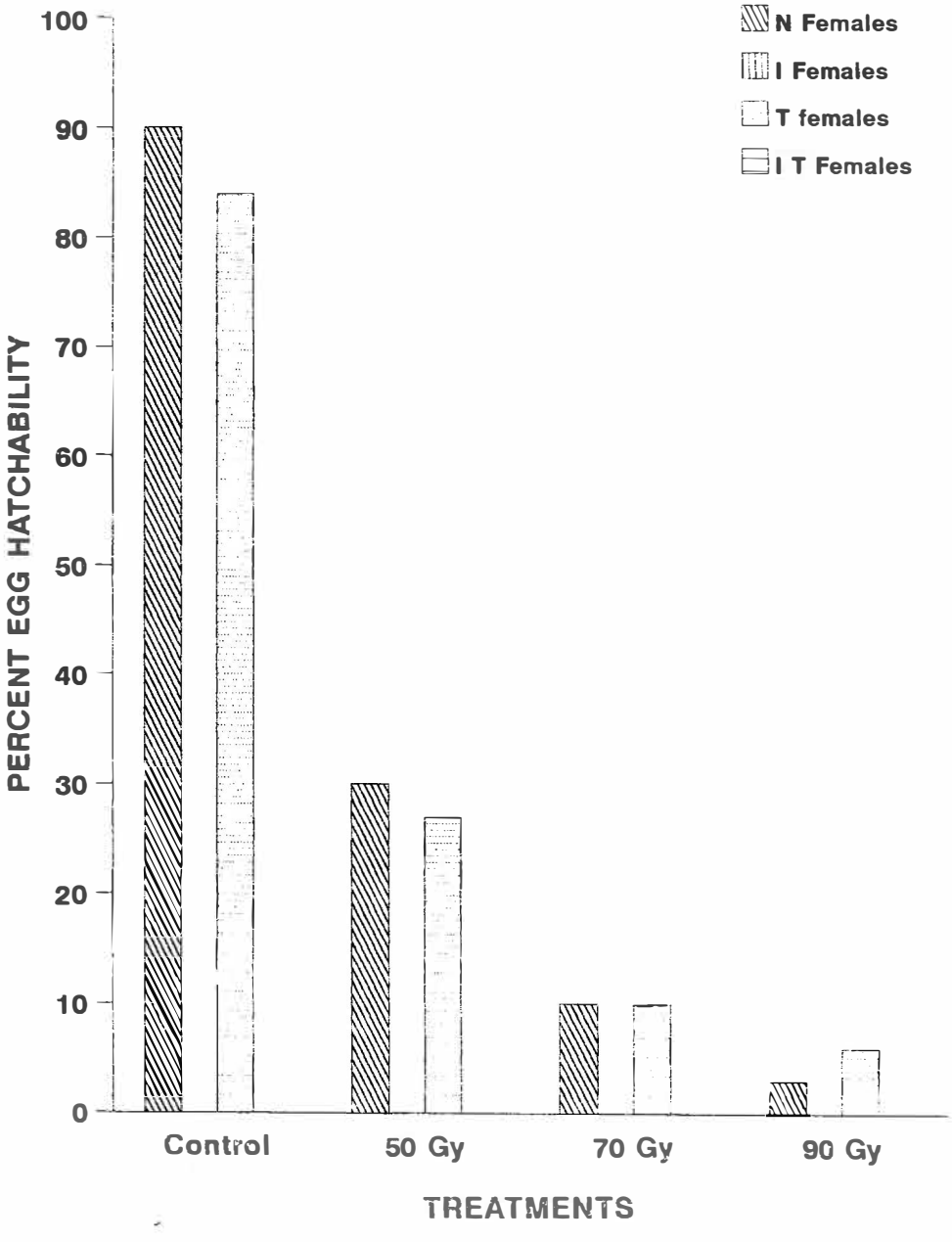

FIG. (2): Effect of irradiating males (as pupae) of Ceratitis capitata with gamma radiation (50, 70, 90 Gray) on egg hatchability when mated with normal, treated (Dimethoate) irradiated (gamma) or irradiated treated females.

gamma doses used. Similar significant decrease in egg hatchability was recorded when treated females were inseminated with irradiated males. Again irradiated and irradiated treated females did not lay eggs when they were inseminated with irradiated males.

The present results on the effects of radiation on male fertility agree with those obtained on the medfly by authors working on the same subject (e.g. Mellado et al., 1966; Hooper, 1971; Schroeder et al., 1973; Wakid, 1973 .... etc.). However, the present results on the effect of dimethoate on egg hatchability disagree with those obtained by other workers on different insecticides (e.g. Albrecht and Sherman, 1987 on the medfly using avermectin, Selim et al., 1987 on ***Spodoptera littoralis*** Boisd., using fenvalerate.). Again, the present results on the combined effects of radiation and insecticides are different from those obtained by Hafez et al., 1987 on ***Culex pipiens*** L. using malathion in the larval stage, and Selim et al., 1987 on ***Spodoptera littoralis*** using LD-10 of fenvalerate. These results indicated a reduction in egg hatchability caused by the combined effect of radiation and the insecticide.

Figs. (3 and 4) show the separate and combined effects of gamma radiation (90 Gy) and dimethoate (o.93 ppm) on mating competitiveness of the irradiated (I) or irradiated treated (IT) males, as percentages observed and expected egg hatch, when confined with irradiated (I) or normal (N) males to compete with normal or treated females at the population ratio of 3:1:1 (I or IT ♂ : N or T ♂ : N or T ♀). From the results presented in Fig. (3) it appears that when irradiated and treated males compete with each other to inseminate normal females the observed egg hatch was higher than that when irradiated male competes with normal males. The competitiveness value was assessed as 0.20 for the former and 0.62 for the later case. Moreover, when irradiated treated males compete with treated males to inseminate normal females, the percentage observed egg hatchability was slightly higher than that when they were competing with normal males. The competitiveness values were, 0.49 and 0.56, respectively.

The data presented in Figure (4) show the same competing populations, in presence of treated females instead of normal females. It was found that when irradiated males competed with treated males to inseminate treated females, the percentage observed egg hatch was higher than when competition was between irradiated males and normal males showing competitiveness values of 0.60 and 0.89, respectively.

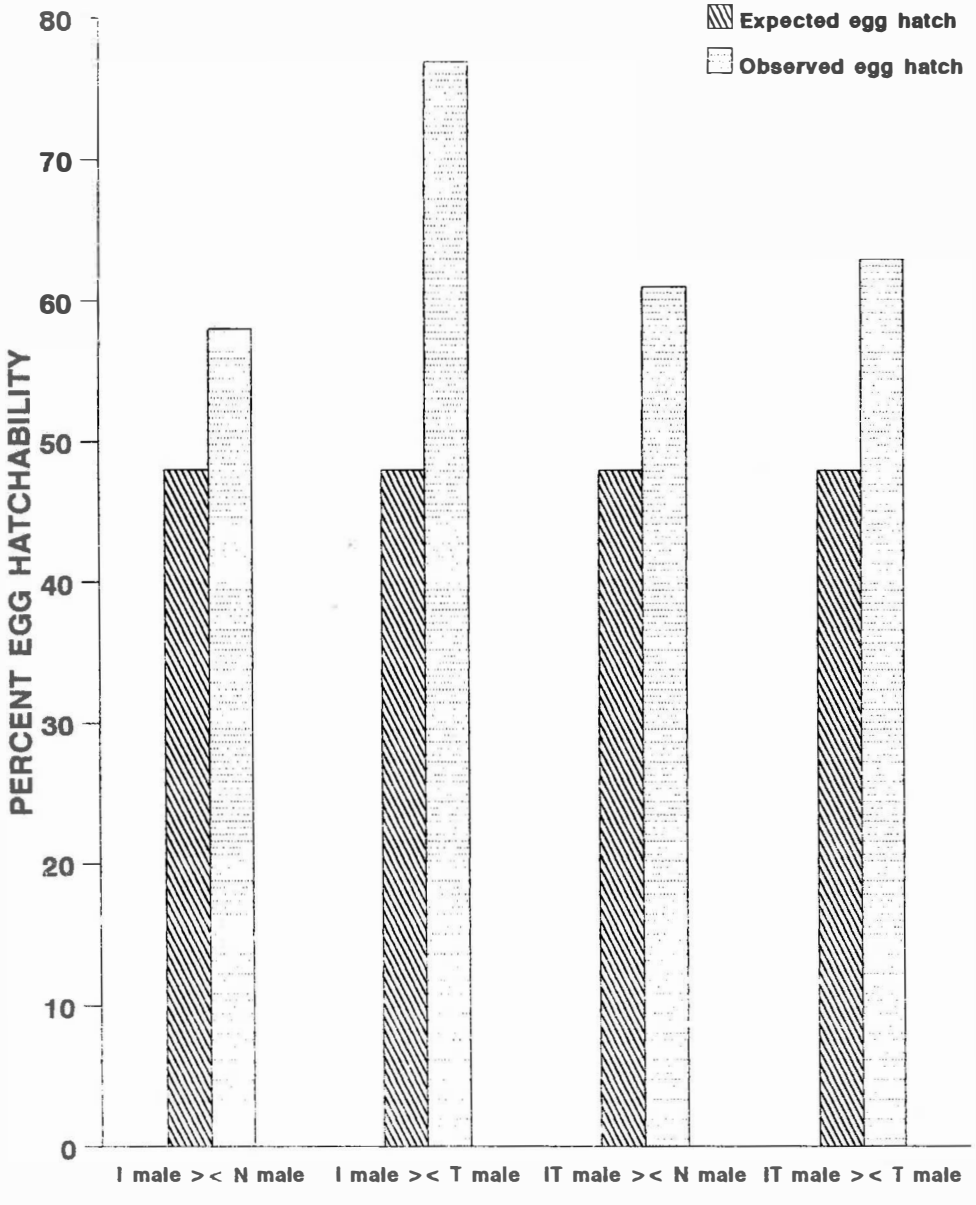

FIG. (3): Separate and combined effect of gamma radiation (90 Gray) and Dimethoate (0.93 ppm) on male mating competitiveness when confined with normwl females.

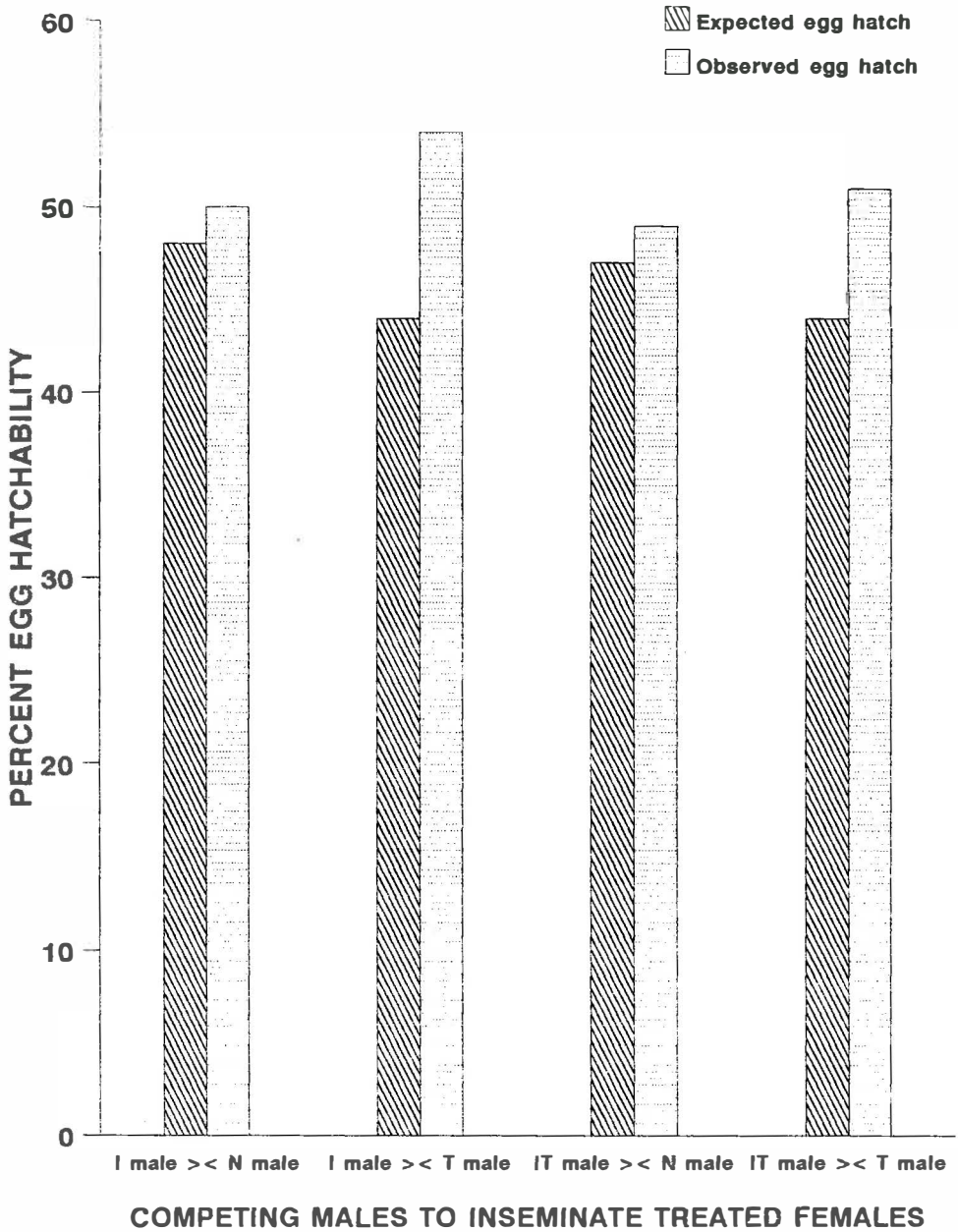

FIG. (4): Separate and combined effect of gamma radiation (90 Gray) and Dimethoate (0.93 ppm) on male mating competitiveness when confined with treated females.

When irradiated treated males were in competition with treated males to inseminate normal females, the competitiveness value was 0.67, while it was 0.90 when normal males were competing with irradiated treated males for mating with treated females.

Competitiveness of irradiated (sterile) medfly males was investigated by several authors (e.g. Chambers et al., 1969; Holbrook and Fujimoto, 1970; Hooper, 1971; Katiyar, 1973; Wakid, 1975; Hashem, 1979). All these authors agreed on the statement that male mating competitiveness was reduced by gamma radiation especially when high doses were applied. This result was arrived at also in the present study. However, a little attention has been paid for studying the mutual effects of insecticides and gamma radiation on the competitiveness of sterile males. For example, Wakid et al., 1987, came to the same conclusion in the present work. They found that the mating competitiveness of ***Culex pipiens*** males decreased significantly especially at high doses of gamma radiation and at high concentrations of dimethoate larvicide. However, Ahmed et al., 1993 recorded that sterile males treated with LC-50 Baygon or Sumithrin and irradiated with 400 Gy were fully competitive, a result which is quite different from ours.

4-Conclusions

Generally the data indicate that the observed percentages egg hatchability of all the competing populations were higher than expected which means that competitiveness of male medfly was affected by both radiation and dimethoate when applied separately or in combination. Moreover, the comparison of mating competitiveness of males inseminating normal or treated females indicated that there was no significant differences between both cases in all combinations except when irradiated and treated males were in competition. This result shows that males from treated population with 0.93 ppm dimethoate were more competitive than untreated males against irradiated or irradiated treated males whether for mating with normal or treated females. The result seems to be unreasonable unless if we assume that males resisting the effect of low dose of insecticide and which could escape mortality should be more stronger than males of the untreated population. The difference between the mating competitiveness of irradiated and treated males to inseminate normal females and that to inseminate treated females was highly significant. In other words, presence of

treated females increased the competitiveness values of the irradiated treated males in all cases of combinations than when normal females were present.

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Session 5

Integrated Control Programme

Chairman: KURT RUSS
Secretaries: E. BOLLER
A. ECONOMOPOULOS

Report on Session 5

KURT RUSS; E. BOLLER; A. ECONOMOPOULOS

The different papers presented in the Session V on "Integrated Control Programs" show that this is only a small sample of a wider array of activities of applied and basic research, and development in this field but certainly more is needed. The discussion showed that the potencial of integrated control of fruit flies is often limited by the high value and high external quality criteria of the fruits and hence by very low economic thresholds. Cost calculations should recieve a higher importance to give more emphasis to the possibilities of implementation of integrated control. It is recommended to this IOBC Working Group to stimulate coordination programs where need to such activities has been identified. Also the concil should be interested to support such activities when other institutions are approached. Dual increased coordinated activities seem to be justified because there is an increasing request to productive procedures that are ecological sound, but still economically feasable.

ELEMENTOS PARA A PROTECÇÃO INTEGRADA EM CEREJEIRA NA REGIÃO DA COVA DA BEIRA, PORTUGAL

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Summary: ELEMENTS FOR INTEGRATED PEST MANAGEMENT OF SWEET CHERRY TREE IN COVA DA BEIRA, PORTUGAL.

The cherry fruit fly (*Rhagoletis cerasi* L.) is considered the major pest problem of sweet cherry trees in Portugal. The use of dimethoate to control this pest could lead to a critical lack of beneficial arthropods.

During three years (1988-1990), a search for mites on leaves was accomplished. Within the phytophagous group, the presence of *Brevipalpus pulcher* Can. & Fanz., as well as of eriophyids, mainly *Rhinotergum cerasifoliae* Petanovic, was highly frequent. We must point out the high number of predator mites (*Amblyseius* spp.) and of indifferent ones (mycetophagous and saprophagous) such as *Orthotydeus californicus* Banks.

The scarcity of insecticide sprays on sweet cherry had not a notorious influence on the acarofauna, even when using an active ingredient such as dimethoate which is very toxic for the predator mites.

1 - Introdução

Este trabalho foi orientado no sentido de racionalizar as acções de combate à *Rhagoletis cerasi* L., tornando-as mais eficientes, e fornecendo informações para a utilização da protecção integrada. A utilização de insecticidas de largo espectro de acção no combate à mosca-da-cereja, como seja o dimetoato, pode levar ao aparecimento de graves desequilíbrios na fauna auxiliar (ACTA, 1990). Sendo o conhecimento da dinâmica dos ácaros fitófagos e predadores uma premissa fundamental para a implementação da protecção integrada (Liguori, 1987), procurou-se obter dados sobre o comportamento bioecológico das espécies fitófagas encontradas, dos seus predadores e de outras espécies que vivem na mesma biocenose, para se poderem programar esquemas de luta química ou de protecção integrada mais favoráveis ao ambiente.

2 - Material e Métodos

As observações foram realizadas, durante três anos (1988-1990), em quatro pomares de cerejeira que se localizam nas freguesias de Alcaide e Alcongosta, no concelho do Fundão (distrito de Castelo Branco). Os pomares são referidos por **B**

(635 m de altitude média), **C** (645 m de altitude média), **D** (650 m de altitude média) e **E** (725 m de altitude média).

Para a determinação das curvas de voo de *R.cerasi* foram capturados os adultos através das armadilhas cromotrópicas amarelas Tipo I da OILB (Russ *et al.*, 1973). Em 1990, foi também utilizada a armadilha de pranchas cruzadas (armadilha «Rebell») para a determinação da curva de voo.

As intervenções químicas realizadas nos pomares, no conjunto dos três anos, são indicadas no Quadro I.

Quadro I - Tratamentos fitossanitários realizados nos quatro pomares, de 1988 a 1990.

Pomar	1988	1989	1990
B	**sulfato de cobre** (queda das folhas)	**sulfato de cobre+óleo** (entumescimento dos gomos); **benomil** (abrolhamento)	**dimetoato** (11/5)
C	**oxicloreto de cobre** (queda das folhas	**sulfato de cobre+óleo** (entumescimento dos gomos); **benomil** (floração); **dimetoato+propinebe** (queda das pétalas)	**dimetoato** (18/4 e 21/5)
D	**Isento**	**sulfato de cobre** (entumescimento dos gomos); **benomil** (floração); **sulfato de cobre** (repouso vegetativo)	**sulfato de cobre** (repouso vegetativo)
E	**Isento**	**oxicloreto de cobre+óleo** (entumescimento dos gomos); **dimetoato** (mudança de cor dos frutos)	**Isento**

Por se terem observado capturas bastante elevadas de alguns auxiliares em 1989, no ano seguinte foram realizadas contagens das formas adultas destes insectos, encontradas nas armadilhas tipo I e Rebell.

A prospecção e flutuação das populações de ácaros foi avaliada em folhas através de amostragens com uma periodicidade quinzenal. Para as amostragens foram marcadas 10 árvores em cada pomar. As colheitas foram realizadas sempre nas mesmas árvores, nos três anos. Esta metodologia de amostragem tem sido utilizada por vários autores em estudos de biologia e dinâmica de populações (Chant, 1959; Genini *et al.*, 1983; Barros, 1987). As folhas foram colhidas à altura de 1.8 a 2.0m na periferia da copa, abrangendo todas as direcções. As amostragens iniciaram-se desde a altura em que já existiam folhas com um certo desenvolvimento, até à sua queda quase total.

3 - Resultados e Discussão

Verificou-se que, em 1989, um elevado número de insectos considerados auxiliares foram capturados nas armadilhas cromotrópicas. A razão deste facto deve-se à atracção manifestada por uma grande quantidade de insectos pela cor amarela (Prokopy & Böller, 1971). As espécies registadas foram *Chrysopa* sp. (NEUROPTERA: CHRYSOPIDAE), *Raphidia xanthostigma* (NEUROPTERA: RAPHIDIIDAE) e *Coccinella septempunctata* (COLEOPTERA:COCCINELIDAE). Além destas espécies, observou-se grande quantidade de pequenos coccinelídeos que não foi possível contar.

A importância destes auxiliares em cerejeira poderá ser bastante grande no combate ao piolho-negro-da-cerejeira (*Myzus cerasi* F.).

No Quadro II podemos observar as percentagens de capturas em relação ao total das datas de avaliação, nos dois tipos de armadilhas utilizados e para os quatro pomares em estudo. Pela análise do quadro referido verificamos a existência de uma maior percentagem de capturas na armadilha Rebell, variando entre 70% para a *R.xanthostigma* e 95% para a *C.septempunctata*, no conjunto dos pomares. O maior número de capturas verificado nas armadilhas Rebell deverá, também, traduzir a maior sensibilidade deste tipo de armadilhas.

QUADRO II - Percentagem de insectos auxiliares capturados nas armadilhas, em 1990.

Auxiliar	Armadilha	Pomar				Total
		B	C	D	E	
Chrysopa sp.	Tipo I	29	0	40	25	24
	Rebell	71	100	60	75	76
R.xanthostigma	Tipo I	22	50	50	0	30
	Rebell	78	50	50	100	70
C.septempunctata	Tipo I	0	0	20	0	5
	Rebell	100	100	80	100	95

A acarofauna da cerejeira, na região da Cova da Beira, incluiu pelo menos 18 espécies de oito famílias diferentes, tendo sido detectadas nove espécies de ácaros fitófagos, cinco espécies de ácaros predadores e quatro espécies de ácaros indiferentes (Quadro III).

Nas Figuras 1 a 3, podemos observar os números de formas móveis de ácaros por folha, nos quatro pomares e nos três anos.

Sendo o dimetoato um dos insecticidas mais tóxicos para a fauna auxiliar, nomeadamente para fitoseídeos, seria de esperar níveis mais baixos para os ácaros desta família, apresentando mesmo o pomar **C** as percentagens mais elevadas destes ácaros.

QUADRO III - Espécies de ácaros identificadas no total de 14800 folhas observadas, de 1988 a 1990 (100 folhas por pomar e por amostragem).

	1988	1989	1990
FITÓFAGOS			
ERIOPHYOIDEA			
ERIOPHYIDAE			
Aculops berochensis Keifer & Delley		x	x
Aculus fockeui (Nalepa & Trouessart)		x	
DIPTILOMIOPIDAE			
Rhinotergum cerasifoliae Petanovic	x	x	x
TETRANYCHOIDEA			
TENUIPALPIDAE			
Brevipalpus pulcher (Can. & Fanz.)	x	x	x
TETRANYCHIDAE			
T.turkestani Ugarov & Nikolski	x		
T.urticae Koch		x	x
T.viennensis Zacher	x	x	x
Panonychus ulmi (Koch)	x	x	x
Bryobia rubrioculus Scheuten		x	x
PREDADORES			
PHYTOSEIIDAE			
Amblyseius finlandicus (Oudemans)			x
Amblyseius stipulatus Athias-Henriot	x		
Amblyseius sp.	x	x	
Typhlodromus sp.		x	
TYDEIDAE			
Homeopronematus anconai (Baker)		x	x
INDIFERENTES			
TYDEIDAE			
Orthotydeus californicus (Banks)	x	x	
Orthotydeus caudatus (Dugés)			x
Tydeus sp.	x	x	
TARSONEMIDAE	x	x	x

Os ácaros fitófagos nunca atingiram densidades de modo a provocarem prejuízos, nem sintomatologia característica. Dentro dos ácaros mais conhecidos como perigosos em fruticultura encontraram-se *Panonychus ulmi* e *Tetranychus* spp. que permaneceram sempre com densidades baixas em todos os pomares.

A acarofauna característica da cerejeira observada na região da Cova da Beira foi constituída por: um tenuipalpídeo, *Brevipalpus pulcher*, que foi a espécie mais frequente na maioria dos pomares e dos anos; os tetraniquídeos, *P.ulmi*, *Tetranychus* spp. e *Briobya rubrioculus*, que só foram detectados a partir de meados de Julho e que nunca atingiram níveis preocupantes, sendo *B.rubrioculus* até muito rara nas folhas; três eriofídeos, *Aculus fockeui*, *Aculops berochensis* e *Rhinotergum cerasifoliae*, que também não apresentaram a característica sintomatologia com folhas prateadas (cor de chumbo); um fitoseídeo, *Amblyseius*

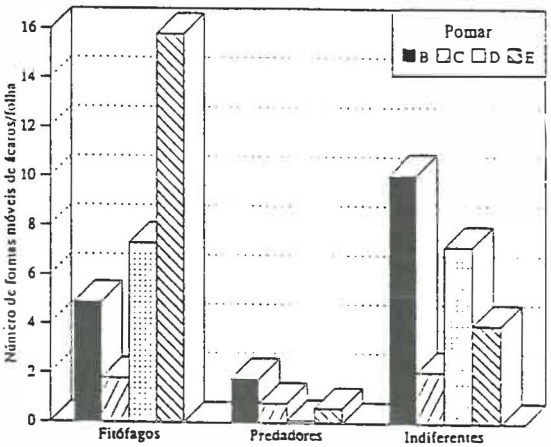

Fig. 1 - Número de formas móveis de ácaros por folha, em 1988.

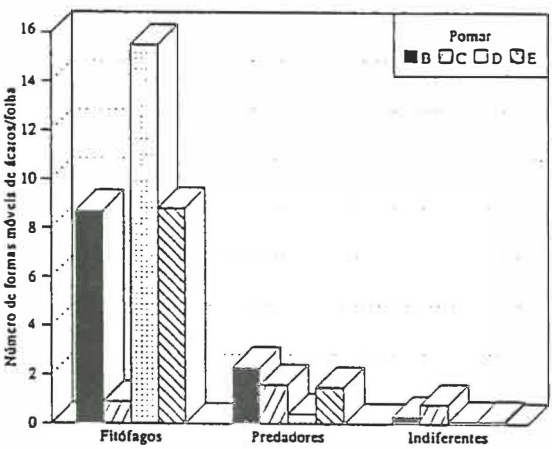

Fig. 2 - Número de formas móveis de ácaros por folha, em 1989.

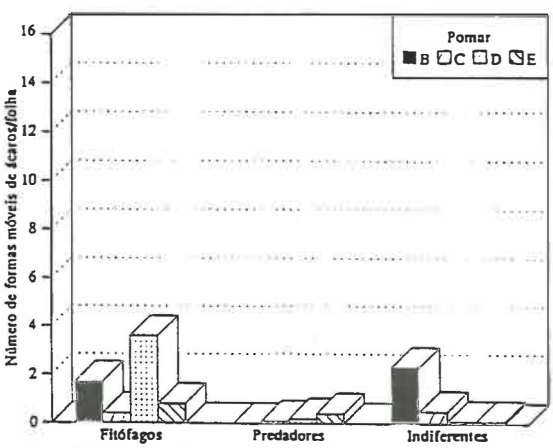

Fig. 3 - Número de formas móveis de ácaros por folha, em 1990.

stipulatus, com níveis consideráveis mas com oscilações muito grandes consoante o pomar; um tideídeo indiferente, *Orthotydeus californicus*, que atingiu, em 1988, níveis bastante elevados.

Na defesa das culturas, segundo critérios de protecção integrada, não se pode ter em conta só a relação fitófago - predador, mas deverá ser melhor avaliado o papel de todos os outros ácaros. A permanência de tideídeos e a alta densidade das suas populações indicam que estes ácaros devem apresentar uma função importante na biocenose da cerejeira. Para Castagnoli *et al.* (1984) essa função pode ser a de predação de eriofídeos e simultaneamente servir de presa alternativa aos fitoseídeos. Esta atitude aliada à capacidade de atingirem níveis elevados de população quando os outros ácaros existem ainda em pequeno número, faz pensar que os tideídeos são um importante factor de equilíbrio.

4 - Conclusões

O uso de armadilhas cromotrópicas amarelas, principalmente das Rebell, para a luta directa contra a mosca-da-cereja poderá ser desaconselhado pelo grande número de armadilhas necessário, cerca de 2 a 10 por árvore (SZO/CSA, 1988) e, consequentemente, pelos elevados quantitativos de insectos auxiliares que são destruídos por serem atraídos para estas armadilhas.

Os tratamentos antiparasitários realizados em cerejeira são normalmente pouco numerosos (principalmente fungicidas), parecendo não terem tido influência de maneira determinante na composição da acarofauna, mesmo usando uma substância activa como o dimetoato, que tem uma acção muito tóxica nos predadores de ácaros.

As mudanças profundas na composição da acarofauna e da sua nocividade, que ocorreram em muitas fruteiras, parecem não terem ainda existido em cerejeira. Estas mudanças resultam da estratégia de luta contra as outras pragas e, principalmente, da escolha dos produtos fitofarmacêuticos que têm efectivamente seleccionado as espécies mais resistentes e conduzido ao desaparecimento de uma grande parte das espécies úteis.

Uma futura intensificação das intervenções fitossanitárias, principalmente para *R.cerasi*, deverá ser acompanhada de estudos da fauna auxiliar, para que se possam conhecer e limitar os efeitos secundários desses produtos. Não está em questão contestar o interesse da luta química que se mantém indispensável, mesmo com os progressos consideráveis da investigação realizada nos outros meios de luta, mas dever-se-á evitar que a acarofauna actual da cerejeira degenere para estados extremamente simplificados e modificados, onde as poucas espécies de fitófagos presentes atingem níveis de nocividade bastante graves.

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ECONOMIC EVALUATION OF FRUIT FLY CONTROL

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Summary

It is important to undertake economic evaluations of the damage caused by fruit flies and the potential benefits of their control prior to the implementation of large scale control programmes. Such evaluations also play a part in the management of ongoing projects and in their final assessment.

Techniques used for the preliminary evaluation of economic losses are discussed, based on work on fruit fly control programmes in the Indian Ocean and North Africa. These cases involve quite different problems. In Mauritius, there is a requirement for import substitution and agricultural diversification which can be improved by controlling a wide complex of flies (at least three of significant economic importance). In the Seychelles, there is a need to increase the supply of local tropical fruit for tourist consumption, which is prevented due to Medfly. In the Maghreb (North Africa), a well established fruit industry suffers considerable losses and high control costs due to Medfly in a market dominated by high quality fruit exporters. All three include significant quarantine considerations. While each of these problems is unique, there are common elements in the economic evaluations, based on an economic model of production, damage and control.

1 - Introduction

All pest management is based on some form of cost benefit analysis. This is often intuitive and confined to individual decision makers, who judge that they have pest problems that worry them enough to justify taking some action. An underlying principle of integrated pest management since the late 1950's has been that there must be a sound assessment or prediction of losses from pests on which to base decisions about appropriate control measures. This is true whether we are planning pest management on an individual farm or for a large area-wide control programme. Unfortunately, many control actions are taken without making even a cursory estimate of potential losses.

Fruit fly control is a particularly important subject for economic assessment, and in many ways it is particularly difficult. It is important because it frequently involves large control campaigns involving public funds, for which there must be accountability. It is difficult for several reasons: Losses are often highly seasonal, and market prices are quite volatile because of seasonal changes in quantity and quality of fruit; losses occur in many different fruit growing sectors in the same area, for instance there may be export producers adjacent to backyard growers, with quite different levels of management; losses may need to be considered over long periods, particularly when determining the benefits of an eradication programme.

During the past three years I have been involved in economic assessments of losses from fruit flies in nine countries of North Africa and the Indian Ocean (Mumford and Butler, 1992, 1993; Mumford and Driouchi, 1993). In each case, a general overall loss assessment was needed on which to base decisions about the feasibility of national or regional fruit fly control programmes. Medfly (*Ceratitis capitata*) was common to all of these countries (apart

from Madagascar (where *Ceratitis malagassa* occurs instead). Reunion and Mauritius also suffer from Natal fly (*Ceratitis rosa*) and Mauritius has, in addition, Mango fly (*Bactrocera zonata*). The flies attack a wide range of fruits at different seasons destined for different markets. There were no readily available sources of information on losses, or indeed fruit production, in these countries.

2 - Methods

The immediate purpose of loss assessments was different for the North African and Indian Ocean programmes. In North Africa it was important to have a method of comparing losses for an area of any given size in which the area and volume of fruit production could be identified. This would enable an assessment of potential eradication and suppression programmes which would operate at different rates (for example 2000 km^2 per year versus 5000 km^2 per year). A flexible system of predicting losses based on area and the type and production of fruit was therefore needed.

In the Indian Ocean, loss assessments needed to produce an overall measure of loss, with some indication of which market sectors (local, import substitution or export) could be improved as a result of control efforts.

In each case the first problem was to determine the level of fruit production. In North Africa, in which there is a large export oriented fruit industry and a substantial local market as well, production statistics were available from Ministries of Agriculture and fruit producers' associations. In Mauritius, a good survey of backyard and orchard trees had been done by the Ministry of Agriculture, but it was necessary to estimate production per tree by species. In the Seychelles, production had to be estimated by calculating annual per capita consumption of fruits and subtracting the volume of fruit imported into the country each year.

For the Maghreb a spreadsheet model was developed with the functions shown in Table I. Fruit production was divided into three main groups of fruit (citrus, pome, stone) and into two production sectors (high and low input). An estimated loss percentage was applied to each category and market prices and distribution then determine the value of loss overall. Losses can be determined using this model's standard assumptions for any zone in which the area and expected yields of fruit can be estimated.

In the Indian Ocean production was estimated from per capita consumption figures based on household surveys conducted by government statistical departments and interviews with hotel managers, since tourist consumption is very significant in some countries of the Indian Ocean. Such household surveys are quite detailed in the Seychelles, identifying fruit consumption by species, whereas in Mauritius and Madagascar all fruit appears as one inclusive item. In the Seychelles, therefore, it was possible to give different loss estimates for each fruit species, while in the other Indian Ocean countries only a global value could be used (which also allowed for an estimate of the proportion of nonsusceptible fruits such as bananas and pineapples).

3 - Results

Table II shows the output from the market and production loss model for Morocco. Table III shows a summary of losses and control costs for all four Maghreb countries based on the same model, with production areas, yields, market prices and exports supplied by local Ministries of Agriculture and fruit producers.

Table I. Functions used in the fruit fly and market model (functions in [] are calculated values, others are input by the user) (Mumford and Driouchi, 1993).

Potential production	■ crop area (hectares by commodity and sector) ■ potential yield (kg/ha by commodity and sector)
Damage	■ [potential production (by commodity and sector)] ■ uncontrolled % Medfly damage (by commodity and sector)
Control	■ % reduction of damage when control is used (by sector) ■ average number of treatments by sector
Control cost	■ unit costs of control ■ average number of treatments by sector
From the inputs and functions above the production of each commodity by each sector is estimated, and losses and control costs are calculated for each commodity and sector.	
Prices	■ farm gate prices at local market quality ■ farm gate prices at export quality
Distribution	■ three markets (fly-free export, export, domestic) ■ export market limits (by commodity) ■ high input commercial production goes to export markets until export limits are reached, any excess goes into the domestic market at local prices ■ all low input domestic production goes into the domestic market
Market response	■ in the export market the prices are considered to be constant regardless of local production, which is assumed to be a small proportion of the total world market ■ the domestic market takes all production in excess of export limits, at constant crop prices
From these functions losses due to Medfly can be attributed to markets and the overall net crop value can be determined.	
Consumption indices	■ [consumption (kg/yr) of domestic produce] ■ [per capita expenditure on fruit in domestic market at farm gate price] ■ [average domestic market farm gate price ($/kg) weighted by commodity]

Table II. Production and loss model output for Morocco (Mumford and Driouchi, 1993).

Maghreb Fruit Fly and Market Model Run: Morocco

PRODUCTION AND CONTROL VARIABLES

Ha ('000)	Total area	106	Production (kg/ha)		Potential production
	High input	Low input	High input	Low input	('000 tonnes)
citrus	65	6	16000	12000	1112
pome	4	11	8000	6000	98
stone	10	10	18000	10000	280

Damage functions (% loss)			Control (% loss saved)		Average loss (%)
	High input	Low input	High input	Low input	despite control
citrus	20	30	80	20	5
pome	30	45	80	20	26
stone	40	60	80	20	22

Sprays per season (average)			Spray cost (@$10/ha)		Total control cost
	High input	Low input	High input	Low input	($ million)
citrus	4	1	2.60	0.06	2.66
pome	5	1	0.20	0.11	0.31
stone	6	1	0.60	0.10	0.7

MARKET VARIABLES

	Price ($/kg)	Market limit ('000 T)	Quantity ('000 T)	Gross crop value ($ million) Market	Subtotal
Fly-free certified exports					
citrus	1.00	0.00	0.00	0.00	
pome	1.00	0.00	0.00	0.00	
stone	0.70	0.00	0.00	0.00	
					0.00
Exports not requiring fly-free certificate					
citrus	1.00	420.00	420.00	420.00	
pome	1.00	0.00	0.00	0.00	
stone	0.70	2.00	2.00	1.40	
					421.40
Domestic market					
citrus	0.10		633.12	63.31	
pome	0.30		72.32	21.70	
stone	0.30		215.60	64.68	
					149.69
				Total	571.09

LOSS AND CONTROL COST SUMMARY

Loss by market ($mn)			Loss by commodity (T'000)	($mn)	Control ($mn)	Loss plus cost ($mn)
Exports (fly-free)						
citrus	0.00	citrus	58.88	5.89	2.66	8.55
pome	0.00	pome	25.68	7.70	0.31	8.01
stone	0.00	stone	62.40	18.72	0.70	19.42
	0.00					
Exports (not fly-free)						
citrus	0.00					
pome	0.00					
stone	0.00					
	0.00					
Domestic						
citrus	5.89					
pome	7.70					
stone	18.72					

Population (mn)	25.60
Consumption (kg/yr)	35.98
Mkt expenditure ($/yr)	5.85
Avg mkt price ($/kg)	0.16

Crop loss ($mn)	32.312
Control cost ($mn)	3.670
Net crop value ($mn)	567.418

Table III. Current annual losses and control costs due to Medfly in the Maghreb countries based on the fruit fly and market model output (US $ million) (Mumford and Driouchi, 1993).

Losses

	Algeria	Libya	Morocco	Tunisia	Maghreb
Citrus	7.39	17.35	5.89	2.81	32.59
Pome	4.25	1.18	7.70	8.42	21.11
Stone	5.69	0.66	18.72	10.63	36.12
Total	17.33	19.19	32.31	21.86	89.83

Control costs

Citrus	0.66	0.66	2.66	0.36	4.34
Pome	0.29	0.16	0.31	0.39	1.15
Stone	0.61	0.21	0.70	0.55	2.07
Total	1.56	1.03	3.67	1.30	7.56

Total losses and control costs

Citrus	8.05	18.01	8.55	3.17	36.93
Pome	4.54	1.34	8.01	8.81	22.26
Stone	6.30	0.87	19.42	11.18	38.19
Total	18.89	20.22	35.98	23.16	97.39

Table IV shows the values of production and imports in the Seychelles. The importance of increasing local fruit production for import substitution is apparent from these figures. In addition to losses to local production, fruit flies also cause additional losses by discouraging local producers from expanding to fill a greater part of the import market.

Table IV. Estimated total consumption of fruit in the Seychelles by source for local and tourist consumption (based on figures from Seychelles Marketing Board, Management and Information Systems Division, and Ministry of Agriculture and Fisheries) (Mumford and Butler, 1992).

	SR million/year	% of total market	% of market sector
IMPORTS	9.9	73	
to tourists	8.8	65	89
to locals	1.1	8	11
DOMESTIC PRODUCTION	2.9	21	
to tourists	0.9	7	31
to locals	2.0	15	69
HOME PRODUCE	0.8	6	
to locals	0.8	6	100
TOTAL	13.6		
Total tourist consumption	9.7	71	
Total local consumption	3.9	29	

Table V shows estimated losses for the five countries in the Indian Ocean, based on the production calculated from per capita consumption and import/export statistics. Losses are expected to be highest in Mauritius, where the Mango fly as well as Natal and Mediterranean fruit flies all attack the fruit. Reunion has a lower level of loss due to reasonably vigorous efforts to control fruit flies, whereas the Seychelles losses appear high due to a total lack of control.

Table V. Estimates of fruit consumption, production, imports, exports and losses from fruit flies in the Indian Ocean region (annual in million ECU) (Mumford and Butler, 1993).

	Consumption	Imports	Exports	Production	Losses	Losses as percentage of production
Mauritius	20.9	4.4	0.8	17.2	3.7	18
Reunion	24.3	12.4	0.9	12.8	1.1 *	8
Seychelles	2.3	1.7	0.0	0.6	0.1	15
Madagascar	22.1	0.0	10.6	32.6	1.7	5
Comores	7.3	0.4	0.0	6.9	0.4	5
Total	76.8	19.0	12.3	70.1	6.9	9.8

* Estimates of the value of losses from fruit flies in Reunion were made by Dr S Quilici, CIRAD, Reunion.

4 - Conclusions

While these estimates of losses from fruit flies are based on quite broad assumptions they have the advantage of using two sets of standardised methodologies that allow cross-country comparisons within the two regions. As a result, they give a reasonable estimate of total losses in each country, and in the case of the Maghreb model they give an indication of losses by crop group. These loss estimates provide a starting point for evaluating the feasibility of control programmes.

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PROGRAMMES DE RECHERCHE ET D'ACTION SUR LES MOUCHES DES FRUITS A L'ILE DE LA REUNION

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Résumé :

Parmi les sept espèces de Tephritidae d'importance économique à l'île de la Réunion, la mouche du Natal, *Ceratitis rosa* Karsch, est de loin l'espèce la plus nuisible aux cultures fruitières.

Depuis 1991, les études visant à une meilleure connaissance de la bio-écologie de ce ravageur et à l'amélioration des méthodes de lutte ont été amplifiées au CIRAD/FLHOR-Réunion.Les recherches visent notamment à mieux comprendre les mécanismes qui guident la femelle dans le choix de son site de ponte, en particulier les stimuli olfactifs. Certaines études sont également consacrées au comportement sexuel du ravageur (phéromones, leks...). Par ailleurs, la biologie d'un parasitoïde ovo-pupal, *Biosteres arisanus* Sonan (Hym.: Braconidae) est également étudiée, en vue d'examiner ses perspectives d'utilisation en lutte biologique.

En matière de développement, une vaste campagne d'information a été engagée dés la fin 1990 afin de promouvoir les méthodes de lutte raisonnée (piégeage sexuel, traitements par taches). La diffusion des matériels et produits nécessaires est assurée grâce à la collaboration d'un large réseau de coopératives agricoles couvrant l'ensemble de l'île.

Suite à l'introduction accidentelle d'une nouvelle espèce (*Bactrocera zonata*) début 1991, un programme d'action a immédiatement été déclenché en liaison avec différents partenaires en vue de son éradication par une combinaison de MAT (Male Annihilation Technique) et de lutte chimique intensive. Un réseau général de surveillance par piégeage sexuel a permis de constater le succès de cette opération, l'espèce étant considérée comme éradiquée depuis février 1992.

1 - Introduction.

Les activités du Laboratoire d'Entomologie du CIRAD/FLHOR-Réunion ont été recentrées ces dernières années sur un groupe de ravageurs d'importance économique majeure, les mouches des fruits. Parmi celles-ci, la mouche du Natal, *Ceratitis (Pterandrus) rosa* Karsch, est de loin l'espèce la plus nuisible aux cultures fruitières de l'île, du fait de sa grande polyphagie et de sa vaste répartition.

Depuis 1991, les activités se sont déployé dans ce domaine selon trois axes :

- des recherches de base, visant à une meilleure connaissance de la bio-écologie de ce ravageur,
- des recherches appliquées, qui ont pour objectif l'amélioration des méthodes de lutte raisonnée contre les mouches,
- des activités de développement, afin d'assurer une large information des producteurs et de diffuser les produits et matériels recommandés en lutte raisonnée.

Par ailleurs, la détection en 1991 d'une nouvelle espèce de Tephritidae dans l'île, *Bactrocera (Bactrocera) zonata* Saunders, nous a conduit à développer très rapidement, en partenariat avec plusieurs autres organismes, une action de lutte spécifique contre cette espèce.

2 - Recherches de base.

On s'est notamment intéressé à la connaissance des mécanismes intervenant dans la sélection du site de ponte chez *C. rosa*, ainsi qu'au potentiel biotique de l'espèce.

Les travaux sur la sélection du site de ponte ont permis dans un premier temps de déterminer l'influence sur ce processus de certains stimuli visuels, notamment la couleur et la taille de l'hôte. On a utilisé dans ce but des dômes artificiels hémisphériques en cire (BOLLER, 1968; PROKOPY & BOLLER, 1971), d'une taille ou d'une couleur donnée, présentés aux femelles dans des expériences de choix. Ces essais portent sur des mouches sauvages de même origine, prélevées au stade larvaire sur des goyaves infestées. Dans la plupart des essais, les femelles utilisées sont naïves et n'ont jamais été en contact avec un substrat de ponte.

On a pu ainsi mettre en évidence un effet significatif de la taille de l'hôte sur la taille de la ponte chez *C. rosa*, les pontes déposées sur les dômes de grande taille comportant plus d'oeufs. On constate également une influence de la couleur sur le choix du site de ponte avec, d'une façon générale, une préférence pour le jaune et le rouge ainsi que, dans une moindre mesure, pour le noir. Ainsi, un plus grand nombre d'oeufs est déposé dans les dômes jaunes, par rapport à l'ensemble des sept couleurs testées. Par ailleurs, l'étude du contraste montre qu'il n'y a pas, chez cette espèce, d'effet significatif de la teinte d'arrière-plan (blanc ou noir) sur le choix de la couleur de l'hôte. De plus, en utilisant des femelles conditionnées à un substrat rouge, on constate que ce conditionnement n'a pas d'effet notable sur leurs préférences ultérieures entre différentes couleurs de substrat.

Les études ont également montré une influence importante des stimuli olfactifs dans le choix du site de ponte par *C. rosa*. On a utilisé pour ces essais des extraits de fruits placés sous les dômes de cire, sans que les mouches puissent entrer en contact avec eux. Ainsi, lors d'expériences de choix incluant des dômes inodores, parfumés à la pêche (pulpe) ou à l'orange (zeste), on constate que la quasi-totalité des pontes sont déposées sur ces derniers.Il semble que cette forte attractivité des dômes parfumés à l'orange soit peu modifiée par l'influence d'autres facteurs comme la couleur ou la taille des dômes, ce qui indiquerait une prépondérance des stimuli olfactifs par rapport aux stimulis visuels.

D'autres fruits, comme le jamrosat, *Sizygium jambos*(L.) Alston., s'avèrent également très attractifs pour les femelles. Le bringellier, *Solanum auriculatum* Ait., se montre moins attractif, alors que le parfum de goyavier (*Psidium cattleianum* Sabine) n'attire pas les femelles. Par ailleurs, un fruit comme le raisin, non attaqué par *C. rosa* dans la nature, provoque une certaine réponse en conditions de laboratoire, les fruits blessés s'avérant plus attractifs que les fruits sains.

Des essais avec différents composés chimiques utilisés isolément (acide acétique, limonène, linalol, fluorène) n'ont montré aucune réponse des femelles aux concentrations utilisées. Plus récemment, les travaux se sont orientés vers l'analyse de l'évolution des émissions volatiles des fruits d'agrumes (mandarine) au cours de leur maturation, en relation avec la réponse des femelles, étudiée au laboratoire,vis-à-vis du parfum de fruits à différents stades de maturité. Ces études, encore en cours, seront poursuivies afin de parvenir à une meilleure connaissance des relations entre *C. rosa* et ses différentes plantes-hôtes.

Des travaux sont également menés sur d'autres aspects de la biologie et du comportement de *C. rosa*. Ainsi, l'étude de l'influence de la température sur le potentiel biotique de l'espèce a été amorçée. Pour les stades pré-imaginaux, on a ainsi pu déterminer la constante thermique et le zéro de développement des stades oeuf et pupe, respectivement 32,8 degrés-jours-11,1°C et 141,5 degrés-jours-12,6°C. L'étude de l'influence de la température sur la fécondité des femelles a montré que celle-ci était maximale à 20°C.

L'étude du comportement de la mouche du Natal en conditions semi-naturelles (grande cage) a été abordée. Elle fournit certaines indications préliminaires sur l'activité et la localisation des mouches sur les plantes au cours de la journée. La description du comportement sexuel, notamment l'appel phéromonal des mâles, montre une certaine analogie avec ce que l'on connait chez *C. capitata*. Toutefois, chez *C. rosa*, l'appel phéromonal intervient en fin d'après-midi, débutant généralement vers

17h. Le phénomène de lek, déjà connu chez diverses espèces de Tephritidae, a par ailleurs été observé pour la première fois chez *C. rosa*.

Une enquête écologique sur les plantes-hôtes des Tephritidae de l'île est par ailleurs en cours depuis deux ans; elle a pour but d'actualiser les données obtenues par ETIENNE (1982), voici une vingtaine d'années. Des prélèvements d'une large gamme de fruits-hôtes sont réalisés dans de nombreuses localités, à différentes périodes de l'année. Cette enquête a permis de découvrir de nouvelles plantes-hôtes et de déterminer les espèces de mouches dominantes sur l'ensemble de la gamme d'hôtes.

En matière de lutte biologique, les travaux ont tout d'abord porté sur l'optimisation des méthodes d'élevage de masse des Tephritidae locaux. Un parasitoïde ovo-pupal, *Biosteres arisanus* Sonan (Hym. : Braconidae), importé d'Hawaii début 93 (grâce à l'amabilité du Dr E. HARRIS, USDA), fait actuellement l'objet d'études bio-éthologiques en préalable à une tentative d'acclimatation.

3 - Recherches appliquées et développement.

Différents essais ont été menés ces dernières années en vue de l'amélioration des méthodes de lutte raisonnée. les premiers ont porté sur l'optimisation des méthodes de piégeage sexuel pour la surveillance des populations. Ces expérimentations ont permis de comparer l'efficacité de différents types de pièges, de diffuseurs d'attractif sexuel ou encore de différentes tailles de plaquette insecticide (DDVP).

Depuis 1991, plusieurs essais en vergers ont permis de confirmer l'efficacité de la méthode des traitements par taches (hydrolysat de protéine + malathion) contre les espèces locales, en vergers d'agrumes et de manguiers. En vue de déterminer les limites de la méthode, des expérimentations ont été conduites sur d'autres espèces fruitières plus sensibles (bibace, goyavier de Chine, pêcher), pour lesquelles les traitements par taches n'ont pas donné pour l'instant de résultats probants. Par ailleurs, plusieurs essais ont été conduits en vergers en vue de déterminer les concentrations optimales d'hydrolysat et d'insecticide : la mortalité obtenue avec différentes concentrations a été évaluée à l'aide de plateaux d'un m² suspendus dans la frondaison des arbres traités.

Parallèlement, un programme visant au développement de la lutte raisonnée contre les mouches, dans tous les cas où elle peut être appliquée, a été initié depuis 1991. Par rapport aux pratiques actuelles des producteurs, ce type de lutte permet une diminution du nombre de traitements (grâce au piégeage sexuel) et du coût de la lutte (réduction des volumes, des quantités d'insecticide et des temps de travaux) qui s'accompagnent d'un moindre impact nocif sur la faune auxiliaire.

Dans ce cadre, une importante campagne d'information (plaquettes, affiches, spots radio et télévision, vidéo), menée en liaison avec la Chambre d'Agriculture de la Réunion, a permis de toucher de nombreux agriculteurs mais également un plus large public. La diffusion des matériels et produits nécessaires (pièges, attractifs...), dont une partie du coût est pris en charge par les Assemblées Locales (Conseil Régional puis Conseil Général), est assurée en liaison avec de nombreuses Coopératives Agricoles. On estime qu'en 1992 les superficies concernées par la lutte raisonnée correspondent à 450 ha de cultures sensibles pour le piégeage sexuel et 115 ha pour les traitements par taches.

Dés la mi-90, un réseau préventif de surveillance par piégeage sexuel au méthyleugénol avait été installé à la Réunion en vue de détecter précocement une éventuelle introduction de *Bactrocera (Bactrocera) zonata* Saunders, espèce d'origine indienne introduite depuis quelques années dans l'île voisine de Maurice. Les premiers individus de l'espèce furent effectivement détectés en février 1991, non loin de l'aéroport. Une action fut immédiatement entreprise en vue d'essayer d'éliminer le ravageur, en liaison avec différents organismes partenaires (Service de la Protection des Végétaux, Fédération des Groupements de Défense contre les Ennemis des Cultures, Chambre d'Agriculture de la Réunion). Le réseau de piégeage sexuel fut fortement renforcé dans la zone et étendu à l'ensemble de l'île cependant qu'une campagne intensive de lutte chimique et de destruction des fruits-hôtes sensibles était entreprise aux abords du foyer. Les captures dans le réseau montrèrent une décroissance

progressive pendant le second semestre 91, puis s'annulèrent en février 92. Seuls deux individus ont été capturés en 1993, sans qu'aucun démarrage de foyer n'ait été observé. On considère donc actuellement que l'espèce a été éradiquée de l'île. Il est toutefois indispensable de maintenir le réseau général de surveillance afin de pouvoir, si nécessaire, intervenir de nouveau très rapidement en cas de nouvelle détection.

4 - Conclusion.

Les travaux en cours depuis quelques années au Laboratoire d'Entomologie du CIRAD/FLHOR-Réunion visent à acquérir une meilleure connaissance de la bio-écologie des Tephritidae nuisibles aux cultures fruitières à la Réunion, et notamment de la principale d'entre elles, la mouche du Natal. Les priorités en matière de recherches futures portent sur les relations insecte-plante, le comportement et les ennemis naturels.

Les méthodes de lutte raisonnée font actuellement l'objet d'un effort de vulgarisation. Elles devront à l'avenir être complémentées par la lutte biologique, qui pourrait notamment s'avérer très utile au niveau des zones-réservoirs de plantes-hôtes spontanées, et par la lutte biotechnique qui fait actuellement l'objet de nouvelles expérimentations.

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MEDITERRANEAN FRUIT FLY EFFECTIVE CONTROL BY COMBINATION OF INITIAL POPULATION REDUCTION FOLLOWED BY SUCCESSIVE RELEASES OF STERILIZED GENETIC SEXING MALES

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Summary

In two separate field application experiments, the Mediterranean fruit fly infestation on citrus and other fruits was effectively reduced, similar to insecticide treated control, by successive ground releases of mass-reared g-sterilized genetic sexing males. Before releases, the wild population was reduced by destruction of host fruits early in spring in the first experiment, or by bait spray on non host tree surfaces, in the second experiment.

1 - Introduction

The construction of "Genetic Sexing" lines of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), Rössler (1979), Robinson et al. (1986), Busch-Petersen (1990), has produced optimism on further expansion and effective application of SIT against this highly destructive, cosmopolitan fruit fly. Laboratory and field evaluation Robinson et al. (1986), Economopoulos (1990), has suggested that such strains could be field applied for an effective medfly control.

As a consequence of the above progress, pilot field testing has been

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undertaken to study control effectiveness of only sterile male releases. In the present experiments a white female pupa strain has been utilized in two different applications, one in Italy and the second in Israel.

2 - Materials and Methods

The first pilot test was applied on the island of Procida, Italy, in 1986. The island is covered by citrus and other fruit tees in systematic plantations or in backyard orchards. It has a total surface of 370 ha (3.7 Km^2) and it is separated by about 2.7 Km of sea from host trees on the mainland of Southern Italy. The second pilot test was applied in the Kibbutz Gvulot Israel, in 1989 and 1990. Gvulot has about 500 ha of citrus and mango systematic plantations and backyard fruit trees. The nearest host tree plantation was at about 6 Km in Kibbutz Zeelim.

In Procida, in February and March sour oranges were collected and destroyed to reduce part of overwintering medfly larvae. Sticky Trimedlure traps were used to monitor newly emerged adults. Sterile males from a white female pupa genetic sexing strain (T-101, Robinson et al. 1986) were mass-reared, sex-separated, and gamma-irradiated at Seibersdorf (IAEA Entomology Unit). Pupae were air-shipped twice a week to Rome and from there transported by car and ferry to Procida, the total transport time lasting about 36 hours. Releases started on April 14 and were terminated on July 7. At the beginning (April) about 1,000 - 2,000 males were released per ha weekly. This number gradually increased to 5,000/ha, bringing the total number of released flies over the entire island to about 20.2 millions. Eight hundred release paper bags were used weekly; 400 for the first weekly shipment

released over half of the island, and another 400 for the second shipment released over the other half of the island. All released flies had been marked by fluorescent yellow powder, except four releases of flies released over half of the island marked red to study survival and dispersal over the other half of the island. The adult population was monitored by "Nadel" Trimedlure taps, activated one day per week, hung in the island and across the 2.7 Km sea strait in the mainland control area. Fruit samples were collected every 7-10 days from the island and the mainland to evaluate infestation.

In Gvulot, to reduce wild population, two applications of malathion bait-spray were applied one or two weeks before releases in April-May in both years. The control area of Zeelim was treated almost weekly by malathion aerial bait-spray from July till October (mangos) and from September till December (citrus). Sterile males from a stabilized white female pupa genetic sexing strain, T:Y (wp) 30 c Busch-Petersen and Kafu (1989), were used on both years. Flies were mass-reared, sex-separated and gamma irradiated again at Seibersdorf. Male pupae were air-shipped once a week (about 2 million male pupae) to Tel Aviv and from there car-transported to Gvulot in the Northern Negev, the total transport time lasting about 24 hours.Thirty five consecutive weekly releases were applied from May till December in 1989 and another 30 from June till December in 1990. Four hundred release bags were used weekly in 1989 and 450 in 1990. Released flies were not marked systematically except certain releases when survival and dispersal were studied, or helicopter releases were compared with ground releases. the adult population was monitored by LADD^{R} sticky colour traps (LADD Inc. Burlington, Vermont, USA). Fruit samples were collected either from under

the trees (fallen fruits usually infested) or from the picking bins during collection in November-January.

3 - Results and Discussion

In Procida, almost no wild flies were trapped till the end of July. Their catches increased gradually in August and reached high levels in September, e.g. over 120 flies per tap per day. In the control fruit orchards, across the sea strait, wild flies were trapped at high numbers since the beginning of July. Similarly, substantial fruit infestation started in Procida only at the beginning of August (about 5%), while in the control, infestation started in the second half of June and it increased to 100% in the second half of August (Fig. 1). Most likely, sterile male releases kept the local wild population at very low level till July. In August, the population had already much increased in the mainland and massive immigration into Procida started, resulting in increased fruit infestation. No releases continued after July because past experience had indicated that immigration of large numbers of wild medflies from the mainland to Procida is unavoidable, because of the close distance and the very high populations built up in the mainland. When red-marked fies were released, the results indicated that more than 80% of recovered flies were usualy trapped within one week from release and that very few of the marked flies dispersed into the other half of the island. For further details refer to Cirio et al. 1987.

In conclusion, the above application of only male SIT resulted in excellent control of local wild flies. Releases were discontinued when massive immigration of wild flies started. The results also indicated that, to maintain

an effective population of sterile males in the field, releases should be applied in a dense release-sites network at least weekly.

In Gvulot, in the 1989 test the wild population increased slightly in June-July and declined thereafter but it did not reach zero level. In 1990, a very small wild population with a slight increase in October - November was detected again. The decline of the wild population in the test area could be also seen from the change in the sex ratio (females/males) which steadily decreased in both years to levels arround 0.01 or 0.02 (Fig. 2). It should be kept in mind that released males always included a small number of laboratory females because the photo-electric separation was never 100% accurate and pupae could not be processed a second time due to increased damage to the flies. In 1989, the total fertile infestation in the test area was 0.07% in the harvested fruits, and 0.13% of total tree fruits,as estimated from infested fruits collected from the ground. The respective %s in the control were 0.04% and 0.004%. In 1990, the respective figures were 0.4% and 0.08% in the test area and 0.6% and 0.21% in the control (Fig. 2). A preliminary test with helicopter release suggested that air release did not harm the flies. For further details refer to Nitzan et al. In press.

In conclusion, in both years a very effective control of medfly wild population was achieved in Gvulot. In fact, in the second year the infestation detected was even smaller than that of the control Kibbutz which received 27 malation aerial bait sprays, i.e. 15 on mangos (Jul.- Oct.) and 12 on citrus (Sept. -Dec.).

The above described only-male SIT applications in two different countries indicated that, releasing only sterile males is a highly effective control method. The fact that no sterile-female stings are produced in the fruits

(cause of secondary infestations) increases enormously the value of the method, especially when fruits are exported under strict inspection procedures to medfly free countries.

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Fig. 1 - Wild medfly adult population and fruit infestation in the test island of Procida and the control area in the mainland (across the sea strait), during the only-sterile-male release application of 1986.

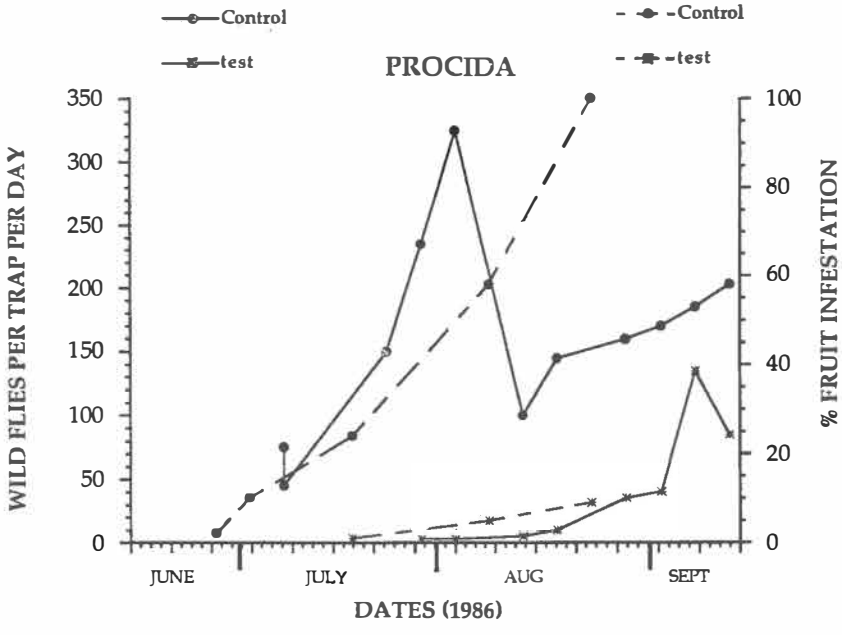
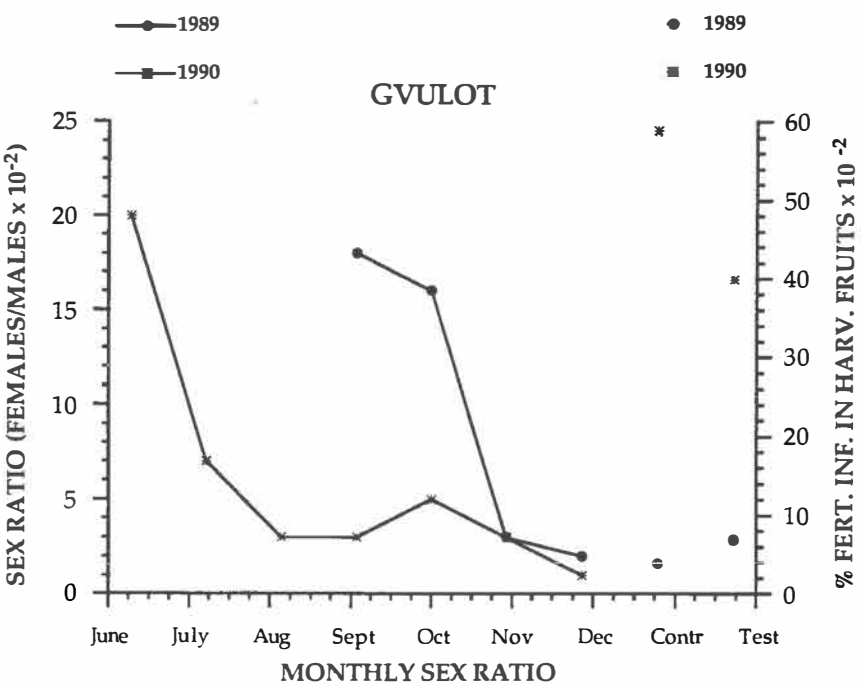


Fig. 2 - Female/male sex ratio in LADD traps and % fertile infestation in harvested fruits in Gvulot (test) and Zeelim (insecticide-treated control) in the 1989 and 1990 only-sterile-male release applications.



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