CROP PROTECTION PROGRAMME

PAKISTAN-UNITED KINGDOM FRUIT FLY PROJECT

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Frontispiece

The cover shows a ber orchard in Khanpur, Punjab, in March 1999, the site of an informal, farmer-managed trial of protein bait sprays (BAT) against fruit flies. This farmer tried a single BAT spray instead of his usual three cover insecticide sprays; the first of these had been ineffective (it may have washed off in rain) and the bait spray was so successful that the other cover sprays were abandoned. The fruit yield was so much greater than normal that the trees' branches were bowed and even broken by the weight. No disadvantages were reported (the trees were due to be pruned anyway). The farmer estimated that production was approximately double the typical one (*i.e.* with the three cover sprays). Visible in the picture is one of the authors (RM) returning empty-handed from checking the plot's four methyl eugenol lure traps, which contained no flies.

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EXECUTIVE SUMMARY

The purpose of this project is the development and deployment in Pakistan of technologies for the monitoring, evaluation and management of fruit flies, which are cost-effective, environmentally benign, and accessible to producers of all types.

The aim of this is to improve the volume and quality of fruit and vegetable production by farmers of all types and classes

It aims to achieve this by experimental comparisons of different field control techniques, in farmers' fields and in liaison with extension services, and focussed research into the distribution, sampling, infestation and control of fruit flies.

At the time of writing, the end of technical assistance in local visits by Imperial College personnel with UK DFID funding, research is continuing in Pakistan for the 1999 season, and Imperial College personnel will assist in the analysis and evaluation of findings and produce a further report.

Control trials in 1998 in three localities found that, despite problems and delays in establishing trials, protein bait sprays may reduce guava losses from 75-83% to 7-27%, equivalent to a rise in fruit production of over 200%, and offer protection at least as good as a programme of intensive cover sprays. Informal farmer-managed trials of bait sprays in guava and jujube reported considerable satisfaction.

In 1999 focussed research projects are investigating the relationship between fly attack and fruit loss, and developing cheap, home-made alternatives to commercial baits, for use by poorer farmers, and lure-impregnated killer blocks to offer a cheaper and durable alternative to hanging plastic traps.

The project has held a training workshop for fieldworkers and researchers, also attended by members of Pakistani officialdom and academia, and held discussions to map out routes to future testing, adaptation and launch of improved fruit fly control technologies.

Attention is drawn to the quarantine problem of the threat to the Indo-Pak subcontinent of the Mediterranean Fruit Fly *Ceratitis capitata*.

The project has evaluated important parameters of fly distributions, and the use of control methods which are cheap, improve control and use reduced quantities of pesticides, which will improve the volume and quality of fruit production, and thus incomes and nutrition, in ways which are cost-effective, accessible and environmentally benign.

FOREWORD

The Pakistan - UK Fruit Fly Project comprises research and development activities carried out by organisations in Pakistan with technical support from Imperial College, London, paid for by the UK Government Department For International Development (DFID). This report marks the end of UK-Government-financed technical assistance in person to the co-workers carrying out research in Pakistan. These researchers will continue work into the future, at least for the 1999 season, and Imperial College personnel will assist in the analysis and evaluation of findings. As a result, this report partly describes work in progress and activities not yet completed. A further report of the conclusions of the 1999 field season will be produced.

Statistical significance tests are throughout given following the conventional terminology:-

ns	$P>0.05$
*	$0.05>P>0.01$
**	$0.01>P>0.001$
***	$P<0.001$

On occasions where the value of *P* is greater than 0.05 but less than or close to 0.1 the precise value of *P* is given. In presenting the results of statistical tests, degrees of freedom are given in [square brackets].

The authors gratefully acknowledge the invaluable support and assistance of numerous people in Pakistan and the United Kingdom who have greatly contributed to the progress of this work. These are too numerous to list, but include the coordinators of the field research work Zafar Ahmad, Karim Nawaz and Arif Makhdum, Drs Ashraf Poswal of CABI Bioscience and John Mumford of Imperial College, the student researchers Qamar Zia, Abdul Hai and Muhammad Azfal, and for their invaluable assistance Mr M. Anwar, Deputy Director (Agriculture), Rahim Yar Khan , Dr Rehmatullah Khan, Director of the Arid Zone Research Institute, Dera Ismail Khan, and Dr Ghulam Mustafa, Director of Entomology at the Ayub Agricultural Research Institute, Faisalabad. Thanks are due to the many farmers who graciously gave up their time, fields and resources to assist in this project. This document is an output from a project funded by the UK Department For International Development (DFID) for the benefit of developing countries. [R6924, Crop Protection Programme].

The opinions and conclusions given are the responsibility of the authors. They do not necessarily reflect the views of Imperial College, CABI Bioscience, or the Department For International Development. Likewise, all errors and omissions remain the responsibility of the authors.

This report and its appendices are all available in electronic form in the word processor programme Corel WordPerfect® by e-mail from the first author at the address above.

SUMMARY OF ACTIVITIES

- The purpose of this project is the development and deployment in Pakistan of cost-effective and accessible technologies for the assessment and control of fruit fly damage, thus increasing the volume and quality of fruit and vegetable production by farmers of all types and classes
- Field assessments of fly damage in 1998 in three localities found the following results:-
    - In Dera Ismail Khan, NWFP, bait sprays reduced guava losses from 75-83% to 7-27%, equivalent to a rise in fruit production of over 200%
    - In Mardan, NWFP bait sprays protected guavas at least as well as a heavy regime of cover sprays with a large reduction in pesticide use
    - In Rahim Yar Khan, Punjab, experiments began too late to prevent guava damage, but reduced fly numbers present in fields
    - Informal farmer-managed trials of bait sprays in guava and jujube reported considerable satisfaction
    - Losses of fruit were considerable in unprotected orchards
    - Infestation distributions differ significantly from the normal, poisson and negative binomial models, and a bimodal distribution is suggested
- In 1999 in four localities, formal trials are under way of bait sprays in melon, mango and ber, and male annihilation technique trials in mango
- A student researcher, developing methods for the assessment of cheap, home-made alternatives to commercial baits, for use by poorer farmers, has found that the assessment technology works as intended, and that baits on natural wood and vegetation are more effective than on sawn timber
- Two student researchers are conducting assessments of lure-impregnated killer blocks and the relationship between fly attack and economic damage
- Meetings with farmers discussed their priorities and perceptions
- Farmer-managed informal trials are assessing bait sprays in melon, plum and apricot
- Economic analysis of the likely effect on fruit prices of possible changes in production patterns indicated that guava prices are relatively stable
- A two-day training workshop for fieldworkers and researchers, also attended by members of Pakistani officialdom and academia, disseminated results and methodology training, and helped establish new objectives
- Discussions with officials mapped out the route to future testing, adaptation and launch of improved fruit fly control technologies
- Attention is drawn to the quarantine problem of the threat to the Indo-Pak subcontinent of the Mediterranean Fruit Fly *Ceratitis capitata*, and a number of pinned specimens of this insect have been delivered to Pakistan for use by quarantine services
- Overall, the project has contributed, and is continuing to contribute, to the development and dissemination in Pakistan of knowledge and expertise to allow the monitoring, evaluation and management of fruit flies, which is cost-effective, environmentally benign, and accessible to producers of all types

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LIST OF APPENDICES

Appendices 1 to 13 give full methodologies, plans of work in progress, and details of research and other activities, and are in a bound volume designed to accompany this report. Appendices 14 to 23 provide basic data used for the analyses described in the report, and are presented as a series of word processor files on aa computer disk designed to accompany this report, in the program Corel WordPerfect®, as files named "PAKFLY##.WPD" where "##" is the appendix number. All appendix files are available on a disk or by e-mail from the first author.

I. BACKGROUND

Fruit flies are serious pests of fruit and vegetables in Pakistan. Their damage has been assessed by an earlier study (Stonehouse, 1997; Stonehouse, Mumford & Qureshi, 1998) and estimated to cause annual losses to Pakistani farmers of over £100 million. This study found widespread concern among farmers of all types, Government officials and fruit traders and distributors about the damage these insects inflict. At the same time, several areas were identified where knowledge was lacking, which might lead to more effective management of these pests. These included the following.

1. Presence and Location of Fly Species

There is a need for more complete information on which flies are found where. In general the distribution of fly species, and their movements within Pakistan with seasonal changes in weather and vegetation fruiting, are incompletely mapped.

There is a particular need for the deployment of trimedlure traps (rare in Pakistan) to keep watch for the Mediterranean fruit fly *Ceratitis capitata*, which is not recorded in Pakistan, but of immense quarantine importance - world-wide it is possibly the single most destructive species; it is found as close as Saudi Arabia, Syria and Turkey, and may be introduced in fruit carried in aircraft (as happened once in India); and it is little looked for, so may be present unobserved. Due to the “ecological island” nature of the Indo-Pak subcontinent, and the currently limited freight traffic through its few land access routes (Khyber and Bolan passes; Karakoram highway; western borders of Burma/Myanmar) the quarantine risk is (a) largely confined to air and sea traffic and (b) best addressed at a regional level by a coherent quarantine programme for the South Asian Region.

2. Abundance and Distribution of Flies

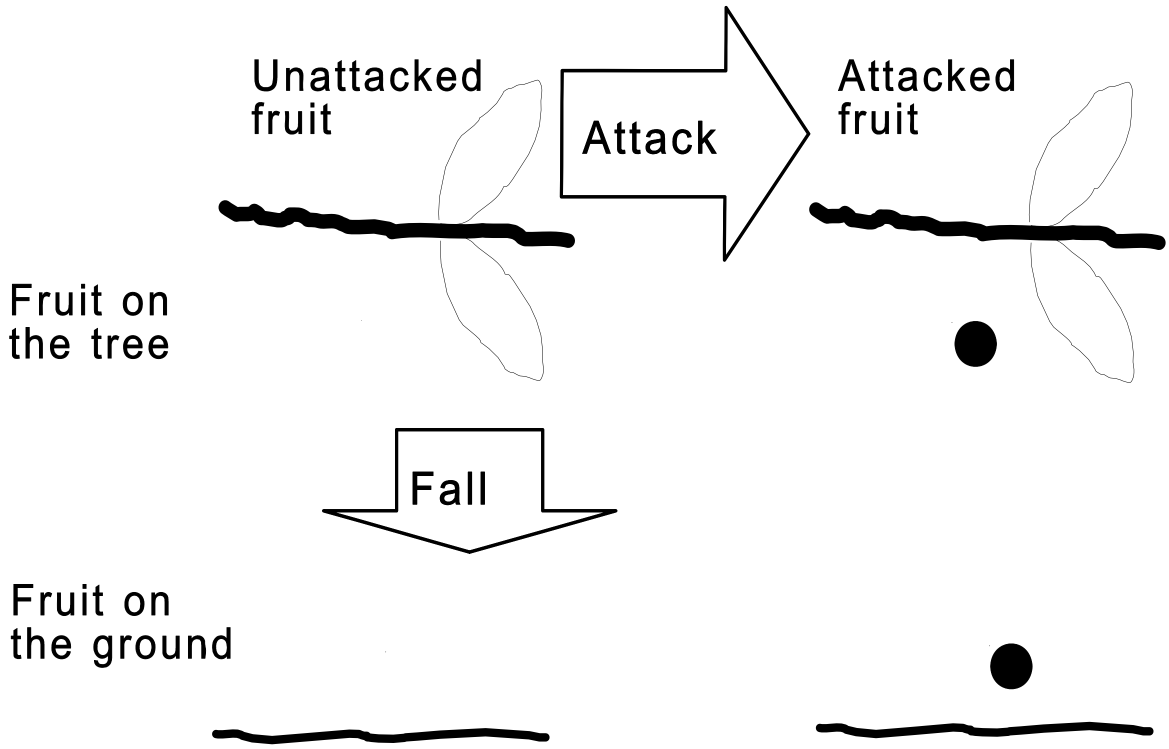
There is a lack of ecological knowledge of how fly populations may be quickly, cheaply and accurately monitored.

First, many studies of fruit flies have relied on catches in parapheromone lure traps, because of their much greater convenience than rearing-out, but they give a poor indication of actual insect density (even with calibration by mark-release-recapture experiments) because the “catchment area” of a trap, within which it will draw males, is highly variable with wind, the presence of alternative attractions *etc.* It is to be hoped that the simultaneous monitoring of fruit infestation by flies and trap catches will allow the relationship between them to be analysed.

Second, infestations may not be the same in fruit on the ground and on the tree, and sampling the former, often preferred for estimation of infestation because farmers are happier to allow researchers to remove fallen fruit, may not be and accurate reflection of the infestation of the latter. The continuous sampling of fruit on- and off-tree for percentage infestation, together with the density of fruit overall on and off trees is desirable, to provide information on the flows of fruits downwards and to the

right between the cells Figure 1, showing how fruit move from the "on-tree; unattacked" category (the only one of major economic value, although fallen fruit are gathered for making pickles) into the other three, with fruit set and production by the tree feeding in new "cohorts" of fruit from the top left corner of the figure. This information will allow accurate assessments of losses from monitoring operations.
A third aspect of importance in sampling is the distribution of infesting larvae among fruits - whether evenly distributed or clustered. The relationship between the size of

Figure 1. Flows of fruit through the stages of falling from the tree and being attacked.



fly populations and the economic damage they do is critically dictated by their distributions among fruit. There may be reasons for attacks to be evenly spread among fruits: some ovipositing fruit flies lay down epideictic pheromones which deter subsequent laying females, although the major pest species, including the ones to be looked for here, do not do this as far as is known. On the other hand, attacks may well be clustered: highly clustered distributions have been found, flies have been observed ovipositing actually into the oviposition scars of previous layers, and interspecific competition between tephritid species may be intense even when fruit attack overall is less than 100%, as when on introduction to Mauritius *Ceratitis rosa* competitively depressed populations of the preexisting *C. capitata*. The distribution of larvae among fruits is critical for the relationship between the average number of flies per fruit (or per unit weight of fruit) and the actual economic loss, which is dictated by the percentage of fruits attacked by one or more larvae. For example, Table 1 shows how the data from two Pakistani trials of MAT may be interpreted - both (Marwat *et al.*, 1992, and Qureshi *et al.*, 1981) giving infestation as numbers of pupae per unit mass of guavas, here converted to numbers per individual fruit on the assumption that a guava weighs 125g. The two data sets - each reduced to single means for unprotected and MAT-protected orchards - are shown with three different assumptions of distribution. The first is “flat” - that each fruit has the average number of larvae for the whole sample. The second is Poisson, as would be expected were the distribution entirely random (using the formula that the frequency of the zero term of the distribution as a function of the mean, *m*, is $1/e^m$ and thus the percentage with infestation by 1 or more is $100*(1-1/e^m)$). The third is an exaggerated and unrealistic “clustered” distribution in which all larvae are found in 20% of the fruit, leaving 80% always uninfested. The final columns show the percentage fruit loss assumed under each scenario in the unprotected and MAT-protected orchards, and the percentage improvement in this represented by the control.

Table 1. Infestation levels of fruit from two Pakistani trials of MAT, inferred with various assumptions as to the distribution of flies among fruit.

Source	Value		Unprot-ected	MAT	improve ment %
Marwat *et al.*, 1992	Number of pupae per 100 fruit		90	68	24
	% fruit loss with assumed distribution:-	flat	90	68	24
		poisson	52	44	16
		all in 20%	20	20	0
Qureshi *et al.*, 1981	Number of pupae per 100 fruit		1071	353	67
	% fruit loss with assumed distribution:-	flat	100	100	0
		poisson	92	71	23
		all in 20%	20	20	0

If the distribution is taken to be flat, the percentage fruit lost is the same as the number of larvae per 100 fruit, to a maximum of 100. If the distribution is taken to be the highly skewed “all in 20%”, reductions in mean numbers of larvae per fruit do not translate into return improvements as the only effect is to reduce the larval load of infested fruits, not their numbers (unless the former drop below 20%). Effectively, relative to the levels suggested by mean figures of larvae per fruit, if distributions of infestation are clustered, percentage infestation levels will be lower, but the improvements recorded by controls will also be lower.

3. Control Technologies - Bait Sprays

In much of the world, protein bait applied in Bait Application Technique (BAT) is regarded as the standard most suitable technique for fruit fly suppression both on and off the farm. BAT sprays, while still containing insecticide mixed with food attractants, have an insecticide dosage of a small fraction of equivalent cover sprays and are positioned to minimise exposure of humans, animals and beneficial insects (the fruits themselves, for example, need not be sprayed). Furthermore, a new development is the possibility of replacing even this small insecticide use with photoactive dyes, which are toxic to fruit flies after ingestion, but harmless to humans and widely used in human foodstuffs and cosmetics (Bersten, 1997).

BAT sprays have been successfully evaluated in Pakistan (Latif *et al.*, 1987) but have not been widely adopted, largely due to concerns over the cost of imported bait. The doses used by Latif and his colleagues, however, were higher than those in many operational programmes, and it is possible that a completely effective bait-+-insecticide mixture would be much cheaper, per hectare, than insecticide cover sprays, particularly when the reduced demands on labour and water (which are problematic in many agricultural areas) are taken into account.

A promising avenue for BAT is the control of melon fly on cucurbits, as shown by the development of this technology by the United Nations Food and Agriculture Organisation in Afghanistan (Stride, 1997). Here *B. cucurbitae* causes considerable damage to melons and other cucurbits, and following a recommendation by staff of this project (JS) in 1996, FAO developed cheap baits for local melon farmers consisting of:-

3l of soup made from 1kg of cheap beef meat (protein source)
150g a.i. malathion w.p. (insecticide)
0.5l filtrate liquid from a large crushed cucumber (cucurbit smell source)
0.5l human or animal urine (ammonia source)

The cucumber extract and urine are mixed together and allowed to stand for two days. The meat is boiled slowly in salted water for 2-3 hours, and removed from the liquid, which is allowed to cool, skimmed of fat and mixed with the fermented urine/cucumber mixture. The mix is stood overnight and mixed with insecticide immediately prior to application. Application is by splashing from brushes early in the morning at approximately 2.5l/ha, and repeated every 10 days. Following understandable resistance from farmers, religious figures and consumers, the urine was successfully replaced with urea, which is used as a fertiliser and is abundantly and cheaply available.

Table 2 shows the results from a trial in Herat in 1996, giving the fruit infestation by flies under bait sprays with two insecticide concentrations, a variety of bait spot spacings, and one area where sprays began before fruit set and one where it was later.

Table 2. Fruit infestation by melon flies with experimental bait spray protection in an FAO trial in Herat, Afghanistan.

Malathion dose	Spray start before fruit set			Spray start after fruit set		
	Spot spacing	Season start	Season end	Spot spacing	Season start	Season end
50	10.5m	0	<1	14m	97	16
100		0	<1		93	15

control		0	60		95	100
50	5 plants	-	-	7.5m	60	15
100		0	0		60	10
control		0	60		60	60
50	1 plant	-	-			
100		0	0			
control		0	60			

These encouraging results prompted the FAO to recommend to farmers the application as above (with malathion WP at 50g a.i./l), at intervals of 10m or 5 plants (whichever is nearer), at ten day intervals and beginning before fruit set. Reports from Afghanistan indicate that the technology is being taken up by farmers and used with great effect. The urine component was unpopular with farmers and consumers, though important as a source of an ammonia smell which attracts flies. For this reason it may be replaced with urea, widely and cheaply available as a fertiliser, and preliminary results from Afghanistan indicate that its effectiveness is not reduced.

4. Control Technologies - Male Annihilation

Experiments in the past summarised in Table 1 (Marwat *et al.*, 1992; Qureshi *et al.*, 1981) have shown that Male Annihilation Technique (MAT) can substantially reduce fly infestation in orchards, but also that infestation is not prevented altogether, and there remains room for further improvement to achieve optimum control.

A possible additional control being evaluated is MAT using dosed wooden blocks instead of the plastic traps currently recommended and used. In Mauritius, a programme designed by Imperial College has successfully maintained low levels of flies by MAT using wooden blocks, soaked in lure and a small quantity of insecticide and nailed or hung to trees. Male flies are attracted to the blocks, feed from their surfaces and, having ingested insecticide, fall dead to the ground. Similar technology in the form of a lure-+-insecticide mix, “eugecide” has been successfully evaluated in the past in Pakistan. In comparison with plastic traps these blocks are:-

- cheaper or home-made
- longer-lasting e.g. in sunlight
- less prone to theft and being blown down
- needing no maintenance or replenishment

It is desirable to evaluate the potential for soaked killer block MAT as a cheaper and more practical alternative to plastic traps.

5. The Distribution of Damage and its Determinants

A further issue of importance is an understanding of how and why insect attack

varies in intensity between space and time. Such aspects include:
- Distribution of infestations on trees - variation with height, aspect *etc*., on several large and small trees
- When infestation densities differ between fruit on the ground and on the tree, which is cause and which effect
- Density dependence of fruit attack, e.g. oviposition deterrence

6. Economic Questions

Several important questions deserve to be addressed on the economic impacts of fruit flies, and also that of possible changes and improvements in their control. These include:
- perceptions of farmers and other stakeholders as to the seriousness of fruit flies as problems, how they have effects (for example by discouraging the sowing of susceptible crops), and how control and management options are viewed.
- differences in the impact of pests, and the relevance and value of controls, between fruit and vegetable production in different sectors (e.g. smallholder, garden, commercial) and within these the fates of products (e.g. home consumption, sale, barter).
- cost estimates of control technologies, and their accessibility to small farmers, particularly protein hydrolysate bait and photoactive dyes, as imports to, and production in, Pakistan.
- calculation of fruit and vegetable supply and demand curves, to estimate price responses to yield increases.
- price elasticities of demand, to examine the effects of fruit fly control on fruit consumption.

II. PROJECT PURPOSE

This project aims to evaluate how the problems of the monitoring, assessment and control of fruit flies may be most cost-effectively addressed, in social and environmental terms as well as economic, by carrying out research to assess the costs of fruit fly damage and their distribution, the most cost-effective control options in various situations, and how ecological and economic factors influence fly damage and the benefits of control.

The basic approach is cost-benefit analysis, in the quantification of the size of losses and the costs and effectiveness of various control options and strategies (including, if the costs of controls exceed their benefits, the option of doing nothing). The primary outputs will therefore be practical and proven information and techniques for the monitoring, assessment and control of fruit flies. These are to be appropriate for the specific geographical, agricultural and economic situations to be recommended to farmers.

The project aims to provide training to agricultural fieldworkers in fruit fly identification, biology and control, both from formal training courses and research and analysis carried out in partnership. It also aims to enter and assist in discussions with farmers, fieldworkers and Government and non-governmental organisation to formulate future plans and activities for the continuation of research if this is found necessary, and for the dissemination and implementation of findings.

III. RESEARCH ACTIVITIES

The research component of this project aims to provide information and understanding of the following areas. The principal research activity, and that which has produced the great majority of the results at the time of writing (July, 1999) is the "Main study" - a systematic sampling of flies from farmers' fields and orchards, in a variety of areas (three in 1998, four in 1999), crops and seasons, and with a variety of control regimes, from none, to farmers' own, to experimental BAT and (in 1999) soaked-wood MAT experimental controls. By quantifying fruit infestation in fields in different areas and under different control regimes, this study serves the purpose of being both a survey of losses in different areas, crops and situations and a comparative trial of control technologies. Other studies remain largely work in progress and their research plans and methodologies are outlined in Appendices 6 to 10.

1. Fly Species Inventory

Flies gathered from different places, times and crops, reared out from fruit and caught in traps, are being retained in permanent reference collections. Research staff have been instructed and trained in the rearing, mounting and preservation of fly specimens.

2. Sampling and the Distribution of Flies in Fruits

Because of the problems of using parapheromone traps to assess fly populations, the main means of quantifying fly infestations in this project is the rearing out of flies from fruits, which provides data on actual infestation and therefore losses of fruit. Both rearing and trap data are recorded together in all sites, to allow comparison of their data. Similarly, the percentage infestation of fruit fallen to the ground (as many surveys record, since farmers are reluctant to allow researchers to pick them) is not the same as that in harvested fruits and therefore economic loss. Again, both are recorded together for comparison, to discern how infestation rates in fallen and hanging fruit differ.

3. Control Evaluations - Bait Sprays

Bait sprays are being evaluated in farmers' fields in the Main study, with results reported below, and also in detailed laboratory and experimental plot studies by the research student Qamar Zia, described in Appendices 6 and 7. Initial work is to confirm the effectiveness and usefulness of bait sprays in principle; subsequent research is to evaluate bait spacing and timing, and the effectiveness of cheap home-made alternatives to bought commercial baits.

4. Controls - Killer Block MAT

Killer block MAT is being evaluated in farmers' fields in the Main study, described in Appendix 5, and also in detailed laboratory and experimental plot studies by the research student Muhammad Afzal, described in Appendix 9.

5. Analysis of the Distribution of Fly Damage and Abundance

These issues are being looked at by the Main study, with results described below, and by research student Abdul Hai, described in Appendix 8.

6. Economic Aspects

Economic questions have been evaluated by the study of statistical records of production volumes and prices, and obtaining data from interviews with farmers, traders, pre-harvest contractors and officials. Appendix 10 describes a simple economic analysis of the elasticity of the relationship between fruit production and its price. Appendix 23 gives full reports of field discussions with farmers, summarised below.

RESEARCH METHODOLOGY -"MAIN STUDY"

The principle questions to ask the data from the main study are as follows:-

- Do controls significantly reduce economic loss and by how much (quantified benefits for cost-benefit analysis)?
- How may fruit fly losses be made apparent? -
    - reduction in volume and quality of harvest
    - percentage infestation of fruit
    - numbers of fruit on trees and the ground, due to accelerated fruit fall
    - delays in fruit development and ripening

(For example, infested fruit allowed to fall off early may be replaced by bringing forward immature fruits which would have otherwise not been developed - this may make good losses in absolute numbers but reduce yields if the later fruit have not enough time to develop fully)

- Do lure trap catches reflect economic losses and if so are they a sufficiently good indicator of these to be used to monitor infestation?
- What is the distribution of damage among fruit? Does this differ between population levels (e.g. between controlled and uncontrolled areas)? How can this knowledge be used to optimise sampling programmes for farmers and researchers (for example the minimum sample size for a certain level of accuracy)?
- What is relative contribution to sampling error of within-tree c.f. between-tree variability? What does this tell us about sampling programmes (e.g. optimum numbers of trees per sample and fruits per tree)?

The core of the main study is rearing-out of flies from fruits to quantify infestation and its distribution. Flies reared from fruits are identified as to species, and also permanently preserved should further identification be found to be useful in future.

The importance of this lies in that advanced taxonomic studies have identified subspecies within species of Pakistani fruit and vegetable flies, yet it is not currently known whether these divisions have any significance for pest damage or control. At the moment this seems unlikely, but it is only prudent to preserve representatives of catches permanently so that, if important differences appear subsequently, catches may be retrospectively assessed for them. A key to distinguish the pest tephritids of Pakistan has been developed (Appendix 1).

The main study is sampling, throughout the year, fruit fly distribution and abundance, and levels of loss. These can be combined with data on fruit and vegetable production and prices to calculate economic damage. Information on what farmers and contractors do or do not do to control pests is being gathered at the same time. Several plots are treated experimentally with unfamiliar treatments by research staff, to evaluate their performance in comparison with local treatments.

This will produce the following benefits:-
1. Identification of which species are present, in what levels of abundance, and whereabouts in space and at which points in time.
2. Accurate costing of losses to the various species, and estimates of the benefits of controls.
3. Baseline of data to be used for the evaluation of any subsequent control effort, as a “control” estimate to which data after control has begun may be compared.
4. Evaluation of ecological parameters dictating pest abundance, distribution and damage. Populations of fruit and vegetable flies characteristically vary in space and time, and the reasons for this are as yet poorly understood. It is hoped that this monitoring programme will allow investigation of this by recording with fly abundance data a brief assessment of the principal environmental and farm variables which may be influential, to provide at least a starting point for analysis of causes and effects. Statistical tests for association of between fly variables, such as fruit infestation, and environmental variables, such as windspeed, shade and control methods, will allow evaluation of which parameters are the key determinants of fly abundance and damage. Similarly, sampling from a wide range of hosts (including irrigated vegetables and wild hosts) will allow identification of potential “reservoir” hosts which allow populations to survive in seasons of low fruit abundance.
5. Training in sampling, identification and data collation. A further benefit will be training and experience for Pakistani fieldworkers in the sampling of flies by various methods, taxonomic identification of species, and data entry to databases and manipulation to extract information.

The accompanying documents explain fully the data gathered and processes followed, being documents developed for research staff for the identification, rearing and preservation of flies, the recording of data in data books produced for the purpose, and applications of bait sprays (a largely unfamiliar technology in Pakistan) as an experimental treatment.

- Appendix 1. *Taxonomic Key to the Fruit and Vegetable Fly Pests (Diptera: Tephritidae) of Pakistan*
- Appendix 2. *The Collection, Rearing, Mounting and Preservation of Fruit and Vegetable Flies (Diptera: Tephritidae)*

- Appendix 3. *The Use of Protein Bait Sprays for Fruit and Vegetable Fly Control*
- Appendix 4. *Gathering Data and Filling Project Data Sheets*
- Appendix 14. *Fruit and Vegetable Fly Infestation Data Book - Tree Fruits*
- Appendix 15. *Fruit and Vegetable Fly Infestation Data Book - Melons*

In 1998 a series of field comparisons were made of Bait Sprays with farmer control (all no-control except *-contractor cover spray) for the suppression of fruit and vegetable flies on farms in the following areas and crops:-

Guava	Dera Ismail Khan (DIK) 1
	Dera Ismail Khan (DIK) 2
	Rahim Yar Khan (RYK) 1
	Rahim Yar Khan (RYK) 2
	Mardan (Mdn) 1*
Persimmon	Mardan (Mdn) 1
Ber	Dera Ismail Khan (DIK) 1
Lufa	Rahim Yar Khan (RYK) 1
	Rahim Yar Khan (RYK) 2

Figure 2 shows the distribution of the research sites.

Figure 2. Location of experimental sites within Pakistan.

IV. OUTPUTS

MAIN STUDY

In not every case could a complete set of data be gathered, but all produced valuable findings.

As corroboration of the common view that the winter guava crop is unattacked because it is so cold, a single fruit sample was taken from Rahim Yar Khan of the winter crop while susceptible and, sure enough, no flies emerged.

1. Harvested Yield

Data were available for the two guava comparisons in Rahim Yar Khan, summarised in Table 3 as average harvest of saleable (pristine or only oviposited) fruit of five trees in each plot, as numbers of fruits, weight of fruits in kg, average weight of a fruit, and sale income of the harvest, with *t*-test results of a comparison of the first two.

It must be emphasised at this point that statistical tests comparing features from two plots do not indicate the general effectiveness of the treatment used. Here and throughout this report samples taken within a plot are used to make statistical inferences about the differences of that plot from another - these samples reflect confidence in the difference *between those two plots*. These differences may be attributable to other features than the experimental controls applied, particularly as fruit flies are notoriously variable in their levels of infestation under natural conditions. To illustrate differences *attributable to controls*, it is necessarily to replicate the plots themselves, as samples representing the wider universe of controls applied or not applied. Thus Table 3 shows that samples taken to indicate differences between the two plots numbered 5 in each; the sample to indicate the effectiveness of the controls numbered two. This point should be remembered in interpreting the statistical results from field comparisons given below.

Table 3. Guava harvests from experimental plots in Rahim Yar Khan, 1998.

Plot	Treatment	Fruit number (#)	Fruit weight (kg)	Mean weight (kg)	Income (Rs)
RYK1	BAT	938.8	83.4	0.088466	3387
	Unsprayed	824.4	72.4	0.086178	2965
	t[8]	2.0480 *P*=0.07474	2.0010 *P*=0.08039		
RYK2	BAT	591.2	57.6	0.095568	2930

	Unsprayed	533.6	56.0	0.104149	2830
	t[8]	1.0923 ns	0.3555 ns		

The bait spray treatment appears to have almost though not significantly increased the total weight of fruit yield and its market value in one plot, though not in the other. Densities of bearing trees were 180/ha (450 in 2.5ha) in RYK1 and 147/ha (184 in 1.25ha) in RYK2, so extrapolation of the income increases from the five trees sampled in each produces benefits for RYK1 of

(3387-2965)*180/5=Rs5192/ha

and for RYK2 of

(2930-2830)*147/5=2940Rs/ha

The lesser efficiency of the sprays in RYK2 may be due to the smaller size of the plot, as bait sprays are more effective over larger areas, or possibly to the presence there from June to November of a cotton intercrop, which may have received some insecticide sprays, although in neither plot were guavas themselves sprayed.

It is hoped that more, similar results next year will (a) be statistically significant and (b) allow fitting of confidence limits to differences between means.

2. Fruit Infestation Levels

The full data as frequencies of infestation of fruits with different numbers of larvae are given in Appendix 16.

i. The distributions of infestation

Figures 3 to 8 show the frequency distributions of pupae, unemerged pupae and adults (all *B. zonata*) collected and reared from guavas from control/check and BAT sites. They are given as the observed distributions and those expected from the negative binomial distribution. The data are converted to logarithms (plus one to avoid deriving LN(0)) as the natural frequencies are overshadowed by the large preponderance of values in the “0" column. The distributions are very highly skewed. Table 4 summarises the means, variances and other parameters of these data.

Table 4. Parameters of the distribution of B. zonata *among guava fruits, 1998.*

Stage	All pupae		Unemerged pupae		Adult *B. zonata*	
Treatment	Check	BAT	Check	BAT	Check	BAT
n (sample size)	730	731	730	731	730	731
_ (sample mean)	2.28	0.70	1.11	0.24	1.45	0.47
s^2	24.20	4.50	3.15	0.76	13.39	2.90

(sample variance)						
k (negative binomial parameter)	0.12	0.069	0.12	0.095	0.096	0.060
χ^2 & [d.f.] (goodness-of-fit to negative binomial)	76.07 [10]	41.86 [5]	12.42 [7]	16.60 [3]	27.20 [7]	11.20 [4]
Significance level (departure from negative binomial)	***	***	*P*= 0.08757	**	***	*

The variances of the samples are much higher than their means, indicating that the samples are not distributed along the lines of the normal or Poisson distributions, but clumped or aggregated. In fact, of 870 guava fruit assessed, 680 had no flies, so that all flies were in 21.84% of the fruit - very close, as it happens, to the “all in 20%” scenario outlined above.

Several mathematical distributions have been used as models for this situation and the negative binomial has often been found to be a suitable model for invertebrate populations. Hence the data were used to estimate a theoretical negative binomial distribution for both control and BAT populations of the number of pupae, unemerged pupae and adult *B. zonata* collected. The methods involved in this process rely on using the sample means and variances and then estimating the negative binomial parameter *k,* which describes the spatial distribution of the populations. There are several methods of calculating *k*. The most efficient method of calculating *k* is that given by the maximum likelihood equation:

$$n \log_e(1 + \frac{\_}{k}) = \sum(\frac{A_{(x)}}{k + \_})$$

Where _ is the sample mean, *k* is the negative binomial aggregation parameter and $A_{(x)}$ is the total number of counts exceeding *x*.

On calculating the value of *k* it can then be used to produce a theoretical negative binomial distribution using the equation:

$$P_{(x)} = (1 + \frac{\mu}{k})^{-k} \frac{(k + \_ - 1)!}{\_!(k - 1)!} (\frac{\mu}{\mu + k})^{\_}$$

A chi-squared test of goodness of fit test is then used to compare the observed and theoretical populations. Agreement to the model is accepted if the calculated value of chi-squared is less than that in tables for the required level of significance and degrees of freedom. The number of degrees of freedom appropriate is the number of classes (*a*) minus one for the constraint of one class by a fixed total sum (Sokal & Rohlf, 1981, p698) and minus another for each of the two parameters (μ and *k*) describing the negative binomial distribution (Sokal & Rohlf, 1981, p713). With the pooling of classes to avoid the occurrence of a class with two few members, the number of classes varied between 6 and 13. The results of these analyses are given in Table 4, expressing statistically the differences which Figures 3 to 8 show graphically, Figures 3 and 4. Numbers of pupae and unemerged pupae in guava fruits from untreated control orchards, showing the distribution found (solid line) and that predicted by the negative binomial formula(dotted line).

Figures 5 and 6. Numbers of adult *B. zonata* emerged from guavas from control orchards, and of pupae from orchards protected by bait sprays, showing the distribution found (solid line) and that predicted by the negative binomial formula(dotted line).

Figures 7 and 8. Numbers of unemerged pupae and emerged adult *B. zonata* from guavas from orchards protected by bait sprays, showing the distribution found (solid line) and that predicted by the negative binomial formula(dotted line).

The observed values of the number of pupae, unemerged pupae and adult *B. zonata* collected from guava are highly clumped but do not adhere to a negative binomial distribution, i.e. a highly skewed and with sample variance significantly in excess of samples means. Figure 9 shows a plot of the natural logarithms of the frequencies of emergences of all pupae emerged from guavas.

In effectively all cases, as can be seen both in Figures 3 to 8 and in the statistical results in Table 4, the observed distribution differs significantly from that expected by the negative binomial formula. Inspection of Figures 3 to 8 makes it clear that the chief difference in most cases is that relatively few fruits contain only one fly - fruits have either no flies or several. This gives the distribution two peaks, making it bimodal, with a large number of fruits uninfested and then a nearly-conventional humped “normal-like” distribution of frequencies around a central value of about nine pupae per fruit. Figure 9 shows this clearly. This distribution appears unusual to statistical theory, being definitely not the negative binomial conventionally used to explain highly skewed distributions, but makes sense in biology, as laying females may be expected to deposit the optimum egg-clutch size per fruit in all fruits. It seems biologically reasonable to infer that a laying female will lay a clutch of eggs at any one oviposition, and that, if the fruit is big enough which guava are, several eggs will be laid. The mathematical description of this relationship will be a more complex matter, but an interesting start point would be to examine the relationship between the upper mode and the size of the fruit.
This distribution implies that the scenarios outlined above in assigning inferred distributions to the data in Table 1 are all wrong, and that a better way to allocate an

average number of flies per fruit to a distribution is to allot them to fruits in batches of nine each until none are left. Thus 870 fruits were examined, 1757 flies were found, and 680 fruits were uninfested. The average number of flies per fruit is therefore 1757/870=2.02, but the average number in infested fruit is 1757/(870-680)=9.25. This distribution obtained in the field was used retroactively to estimate this effect in the two old data sets of the Marwat and Qureshi guava MAT studies shown in Table 1. Fitting this new distribution to the data obtains the results shown in Table 5.

Table 5. Infestation levels of fruit from two Pakistani trials of MAT, inferred with the distribution of flies among fruit found in the field.

Source	Value	Unpro-tected	MAT	% Impro-vement
Marwat *et al.*, 1992	Number of pupae per 100 fruit	90	68	24
	% fruit loss with assumed distribution	10	7.5	25
Qureshi *et al.*, 1981	Number of pupae per 100 fruit	1071	353	67
	% fruit loss with assumed distribution	100	39.2	60.8

It is clear that the MAT with this assumption provides much better protection than with any of the assumptions preliminarily considered and outlined above.

ii. Percentage losses to fruit flies

The full tables given in Appendix 16 give the full infestation figures in protected and unprotected fields. These tables are unwieldy and hard to interpret, and so the results are summarised Table 6 as the percentage of fruit on the tree from which a fruit fly pupa emerges, which are those economically lost to the farmer (those with “exit holes” are omitted as these may be due to all sorts of things, such as bird and beetle damage). Presented in Table 6 are the values for guava only - those for ber, persimmon and lufa show little of interest, implying an absence of differences attributable to treatments, and are in Appendix 17. Guava collections in several locations were hampered by logistical problems, and difficulties in rearing conditions sometimes led to the complete absence of emerging larvae, implying mortality of all larvae in the fruit, and so many data sets are incomplete.

As the data are now frequencies cast in contingency tables, the statistical test used for analysis was the *G*-test for comparisons of frequencies. This little-known test is similar in use to the more familiar XxY contingency chi-squared test, but allowing analysis in more than two directions, as here in 2x2x2 three-dimensional tables (Sokal & Rohlf, 1981, p750). This allows analysis of differences between

- damage - good versus attacked fruits
- treatments - BAT versus control
- positions - tree versus ground

and of all two- and three-way interactions between them

Full output from the *G*-analysis is complex, and the full tables are reproduced in

Appendix 18. Table 6 gives only those values for 3-way interaction and for those between infested/uninfested fruits and bait-sprayed/check plots (the presumed effects of sprays) and between infested/uninfested fruits and those on the trees or ground (presumed difference in infestation between fruit on trees and on the ground, and for three-way interaction between all three variables. (Note that the third two-way interaction, between numbers of fruit in treated/check plots and fruit on trees/ground, is invariably insignificant as the sampling design constrains these categories to have always the same number of fruit - 30 - in each cell). Values given here for two-way interactions are discounting the three-way interaction term to give the effects of the two-way alone; full figures are given in Appendix 18. The *G*-values given have been treated with the Williams correction to improve the fit of the statistic to the chi-squared used as test distribution (Sokal & Rohlf, 1981, p736).

Table 6a. Percentages of fruit infested by flies under different control regimes - Dera Ismail Khan, 1998.

D.I. Khan 1		Date	1	2
Percentages damaged	Fruit on trees	BAT	3	7
		Unsprayed	10	83
	Fruit on ground	BAT	17	27
		Unsprayed	20	60
G, [d.f.] & significance	damage*treatment*position [1]		0.499ns	8.330**
	damage*treatment [1]		0.682ns	37.972***
	damage*position [1]		3.779ns	-0.163ns
D.I. Khan 2		Date	1	2
Percentages damaged	Fruit on trees	BAT	3	27
		Unsprayed	10	77
	Fruit on ground	BAT	10	17
		Unsprayed	20	93
	damage*treatment*position [1]		0.064ns	3.950ns

	damage*treatment [1]	2.191ns	55.187***
	damage*position [1]	2.191ns	0.119ns

Table 6b. Percentages of fruit infested by flies under different control regimes - Mardan, 1998.

Mardan		Date	1	2	3	4	5
Percentages damaged	Fruit on trees	BAT	17	23	13	43	17
		Full spray	20	23	33	17	40
	Fruit on ground	BAT	63	17	27	43	27
		Full spray	67	13	33	27	47
G, [d.f.] & significance	damage*treatment*position [1]		0.008 ns	0.074 ns	0.958 ns	0.510 ns	0.151 ns
	damage*treatment [1]		0.172 ns	0.052 ns	2.674 P=0.102	6.360 *	6.403 *
	damage*position [1]		27.496 ns	1.319 ns	0.658 ns	0.340 ns	0.977 ns

The following observations may be made:-
1 - the BAT sprays significantly reduce percentage infestation in many cases - in the clearest case, guava in D.I.Khan, the percentage infestation was 7-27% in protected plots, and 77-83% in unprotected, equivalent to an increase in fruit production of 200%
2 - the reduction is not always as large as would be liked. The exceptions may often be attributed to specific factors - the coordinator of the D.I.Khan guava trial believes the application was made in good time, and it was successful in the sense that it at least matched if not bettered the level of protection of the heavily sprayed farmer check plot adjacent; the guava application in R.Y.Khan, on the other hand, is felt by its manager to have started too late (as Figure 2 shows, D.I.Khan is substantially further north than R.Y.Khan, and the season starts later).
3 - percentage infestation often appears to be higher in fruit on the ground than on the tree - this was not statistically confirmed, but the phenomenon in general was confirmed by many fieldworkers and farmers familiar with patterns of fruit fly attack.

3. Fruit Production Volume

That infestation in fruit on trees is less than those on the ground is well known, and presumed to be because attacked fruit are likelier to fall, rather than fallen fruit likely to be attacked. This gives rise to the suspicion that percentage infestation data like those above will not fully capture economic losses as, if attacked fruit have fallen off, the absolute number of fruits on attacked trees may also be lower. For this reason the absolute numbers of fruit on both trees and the ground were also recorded. These data are given in Table 7. Fruit density data were converted to densities per unit volume of tree canopy (for fruit on trees) or per unit area of ground below the canopy (for fallen fruit), to discount differences in tree size between plots. Estimates were made of the numbers of fruit (in each of four age classes, broadly characterised as Undersized, Green, Ripening and Ripe) on each tree sampled, and the area of ground covered by the tree and its height also estimated; the numbers of fruit were divided by a crude estimate of tree volume, obtained by multiplying the height by area estimates, to obtain an estimated density per cubic metre of tree canopy. Fruit on the ground were counted in three randomly-thrown square-metre quadrats beneath each tree, and the average of the three throws was used as an estimate of fruit density per square metre beneath each tree. As so often the data for lufa, ber and persimmon showed no patterns of significance to suggest treatment effects, and are given in Appendix 19.

Statistical treatment of the results was by analysis of variance (ANOVA), as two-way ANOVAs of differences between fruit densities (a) between treated and untreated plots and (b) between fruit on trees and on the ground. The full ANOVA tables from these comparisons are given in Appendix 20; the *F*-values obtained, and their degrees of freedom and significance levels, are in Table 7.

Table 7a. Densities of guava fruit under different control regimes - Dera Ismail Khan, 1998.

Date				1	2
DI K 1	Density of fruits	Fruit on trees (/m^3)	BAT	6.41	0.66
			Unsprayed	10.87	0.96
		Fruit on ground (/m^2)	BAT	1.33	7.33
			Unsprayed	2.07	13.87
	ANOVA *F*, [d.f.] & significance	treatment [1,16]		3.859ns	34.809***
		position [1,16]		27.655***	286.018***
		treatment x position [1,16]		1.988ns	28.963***
	Density of fruits	Fruit on trees (/m^3)	BAT	4.49	0.58

			Unsprayed	4.32	0.82
		Fruit on ground (/m^2)	BAT	5.20	4.80
			Unsprayed	1.47	10.73
	ANOVA *F*, [d.f.] & significance	treatment [1,16]		8.038*	17.111***
		position [1,16]		2.421ns	89.696***
		treatment x position [1,16]		6.656*	14.535**

Table 7b. Densities of guava fruit under different control regimes - Rahim Yar Khan, 1998.

Plot (date 2 only)			RYK1	RYK2
Density of fruits	Fruit on trees (/m^3)	BAT	0.07	0.05
		Unsprayed	0.08	0.05
	Fruit on ground (/m^2)	BAT	1.00	1.60
		Unsprayed	2.13	2.80
ANOVA *F*, [d.f.] & significance	treatment [1,16]		8.642**	9.851**
	position [1,16]		59.241***	128.187***
	treatment x position [1,16]		8.401**	10.117**

Table 7c. Densities of guava fruit under different control regimes - Mardan, 1998.

Date			1	2	3	4	5
Density of fruits	Fruit on trees (/m^3)	BAT	71.23	83.02	69.56	35.03	11.10
		Full spray	68.10	63.14	53.98	24.77	11.14
	Fruit on ground (/m^2)	BAT	2.00	7.13	6.13	4.73	3.93
		Full spray	1.80	5.27	4.93	4.20	4.13
ANOVA	treatment [1,16]		0.0369 ns	0.711 ns	0.751 ns	1.700 ns	0.005 ns

	position [1,16]	60.976 ***	26.876 ***	33.750 ***	37.771 ***	17.596 ***
	treatment x position [1,16]	0.029 ns	0.487 ns	0.551 ns	1.380 ns	0.002 ns

These results show no evidence that protected plants have more fruit on them than unprotected, but also it appears that there are more fruit on the ground in unprotected than protected fields. This pattern has been repeatedly referred to as an impression widely gained in observing fruit fly attack in the field. This was confirmed by *t*-test comparisons between the treated and untreated plots of the densities of fruit on trees and on the ground separately, with the results in Table 8.

Table 8. Outcome of t-test comparisons of fruit on trees and on the ground. Degrees of freedom in all cases was eight.

D.I. Khan 1	Date 1	Date 2
Fruit on trees	1.871ns	1.475ns
Fruit on ground	1.519ns	4.097***
D.I. Khan 2	Date 1	Date 2
Fruit on trees	0.619ns	1.735ns
Fruit on ground	2.458*	3.699**
R.Y.Khan (date 2 only)	RYK1	RYK2
Fruit on trees	1.031ns	0.106ns
Fruit on ground	3.050*	3.101*

This opens the possibility that plants are compensating for infested and fallen fruit by increasing production of fruit, or ceasing to abort a surplus of potential fruit which normally would not ripen but are kept back in reserve for just such a contingency as this. This being the case, the “replacement” fruit being developed to replace those lost may be expected to be behind their fellows in development, and so unprotected fruit may be later in developing than protected, and this late development may lead to economic losses if fruit are not fully grown and matured by harvest. As a result, the development stage of the fruit was also compared.

4. Fruit Development and Maturation

These data are given as full frequency tables in Appendix 21, and summarised in Table 9 as the average fruit development stage for each treatments at each date, on the following score:-

1=small green fruit
2=full-sized green fruit

3=ripening fruit
4=ripe fruit

Table 9. Stages of fruit maturation under different control regimes, 1998.

Fruit	Site	Treatment	1	2	3	4	5
Guava	DIK1	BAT	2.10	2.18			
		Untreated	2.01	2.50			
	DIK2	BAT	1.85	2.50			
		Untreated	2.16	2.39			
	RYK1	BAT	2.50	3.45			
		Untreated	2.81	3.61			
	RYK2	BAT	2.50	3.69			
		Untreated	2.89	3.56			
	Mardan	BAT	1.08	1.56	1.72	1.83	2.55
		Cover spray	1.07	1.55	1.97	2.08	2.52
Persi-mmon	Mardan	BAT	1.09	1.17	2.37	3.45	
		Untreated	1.11	1.19	2.38	3.50	
Ber	DIK1	BAT	2.00	2.20			
		Untreated	2.15	2.43			
Lufa	RYK1	BAT	2.76	2.73			
		Untreated	2.84	2.85			
	RYK2	BAT	2.72	2.70			
		Untreated	2.70	2.75			

In fact, no differences in development rates between treated and untreated areas are apparent. It looks most unlikely that there are any differences between the treated and control plots in terms of speed of fruit development when measured in this way. If anything, it appears that development is accelerated in the unprotected plots, and indeed this is often a characteristic of fly-infested fruit. These data were compared statistically by means of two-way analyses of variance, comparing densities of fruit in the four age classes between treated and untreated plots, but no significant interactions were found. The output of these analyses are given in Appendix 22. (An alternative might be to compare the age classes as on a scale, but , as they are not true “interval” variables such as counts or measurements, as might be evaluated by regression or correlation coefficients, but rank-ordered categories, they would best be compared by a test for ordered categories such as the Gamma statistic (Siegel & Castellan, 1988, p291)).

5. Trap Catches

Trap catch data are given in Table 10, as catches per day of exposure in the field.

Table 10. Catches per trap per day of adult flies in methyl-eugenol baited traps in field plots, 1998.

Fruit	Site	Treatment	1	2	3	4	5
Guava	DIK1	BAT	8.75	15.00			
		Untreated	11.75	24.09			
	DIK2	BAT	3.45	12.50			
		Untreated	10.34	19.00			
	RYK1	BAT	40.97	11.22			
		Untreated	94.83	44.72			
	RYK2	BAT	22.37	9.61			
		Untreated	64.63	19.35			
	Mardan	BAT	6.20	1.45	0.31	0.38	0.17
		Cover spray	4.60	2.90	0.53	0.66	0.29
Persi-mmon	Mardan	BAT	1.24	0.29	0.60	1.29	
		Untreated	0.94	0.65	0.93	2.29	
Ber	DIK1	BAT	0.17	0.53			
		Untreated	0.83	0.20			

i. Trap catch differences between protected and unprotected plots

Catch data were examined to show differences between treated and untreated plots. These data do apparently show that trap catches are lower in bait-protected than in control plots - which is particularly reassuring in the R.Y.Khan guava plots (where BAT is believed to have started too late to protect fruit from oviposition, but does seem to have had the effect of reducing numbers of flies on the wing subsequently in the season) and in the Mardan guava plots (where the “check” plot was cover-sprayed, but lower catches in the BAT-treated plot would imply control even superior to these sprays). This was confirmed by statistical comparison, whereby the catches of the trap pairs in the five guava plots over all samples taken (total number of trap pair catches: thirteen) were compared by related *t*-tests and showed a significant difference (t=2.515[12]*). (Differences were not significant between treatments in the persimmon or ber plots). When sequential catches on the same plot were not treated as replicates (as is correct as these are not mutually independent) and sequential catch data were pooled for each plot, differences were not significant, either for the five guava plots (t=2.051[4]; P=0.110) or for all seven plots together (t=1.892[6]; P=0.107). The presence of fewer flies in protected plots is implied but not demonstrated.

ii. The relationship between trap catch and infestation data

A further objective was to associate trap catch data with percentage losses in the same plots, to ascertain if the former may be used as convenient predictors of the latter. This was done for guavas only. The data for trap catches from one time interval were compared with those for emergence of pupae from fruit gathered at the end of the same interval. It may be that a better fit would be obtained between data from different time intervals for trap and fruit emergence data, to allow for the development of generations in different media, but the same interval was used following the argument that, as the traps are emptied at the same time as the fruit collected, the preceding interval should record the laying activities of those adults flying about in the same period.

The larval infestation figure used for comparison was the total numbers of larvae per tree. This was obtained by multiplying the volume of fruit recorded on trees and on the ground by the frequencies of pupae emerging in the samples taken at the same times (fruit which were exit-holed when collected were excluded). These totals are given in Table 11.

Table 11a. Estimated total numbers of fly larvae per tree - D.I.Khan, 1998

D.I.K.1	Date:-	1	2
Plants	BAT	21350	15680
	Unsprayed	42600	221805
Ground	BAT	27692	30510
	Unsprayed	3078	281392
Total	BAT	49042	46190
	Unsprayed	45678	503197
D.I.K.2	Date:-	1	2
Plants	BAT	17060	31820

	Unsprayed	50810	112575
Ground	BAT	1128	20177
	Unsprayed	6714	246874
Total	BAT	18188	51997
	Unsprayed	57524	359449

Table 11b. Estimated total numbers of fly larvae per tree - R.Y.Khan, 1998

R.Y.K.1	Date:-	1	2
Plants	BAT	-	-
	Unsprayed	3526	248
Ground	BAT	150	-
	Unsprayed	85404	3837
Total	BAT	-	-
	Unsprayed	88930	4085
R.Y.K.2	Date:-	1	2
Plants	BAT	0	-
	Unsprayed	1150	-
Ground	BAT	5	1004
	Unsprayed	14845	5096
Total	BAT	5	1004
	Unsprayed	15995	5096

Table 11c. Estimated total numbers of fly larvae per tree - Mardan, 1998

Mardan	Date:-	1	2	3	4	5
Plants	BAT	0	125235	68278	94920	11415
	Unsprayed	0	77248	103680	26912	27222
Ground	BAT	0	3237	2433	3440	2225
	Unsprayed	0	1347	2359	2180	3000
Total	BAT	0	128472	70711	98360	13640

	Unsprayed	0	78595	106039	29092	30222

Statistical treatment was by regression comparison of the 'Total' values for larvae present with the trap estimates of adult density at the time produced very poor associations. A regression of the two sets of values obtained an *R*-squared value of 0.001442; an attempt to allow for differences by including zone, treatment and date as further independent variables in a multiple regression obtained an *R*-squared value of 0.225580; the omission of suspicious data, namely all values of zero emergence which may be attributable to mortality in the rearing rooms, obtained an *R*-squared of 0.213079. Observation and analysis of this relationship will continue, but it currently seems most unlikely that trap catches may be used as reliable predictors of fruit attack rates.

6. Total Production of Useable Fruit

The final comparison is of the total useable fruit on trees at the last count - the closest value to the harvest itself. This value should provide the most accurate predictor of harvestable yield and therefore farmer income, and is the total load of fruit on trees minus that fraction which is attacked. These totals are shown in Table 12.

Table 12a. Estimates of total loads of fruit per tree - guavas in D.I.Khan and R.Y.Khan, 1998.

Farm	Date:-	1	2
DIK1	BAT	4128	429
	Unsprayed	2840	133
DIK2	BAT	3412	315
	Unsprayed	4065	111
RYK1	BAT	-	38
	Unsprayed	32	30
RYK2	BAT	-	0
	Unsprayed	40	0

Table 12b. Estimates of total loads of fruit per tree - guavas in Mardan, 1998.

Date:-	1	2	3	4	5
BAT	4412	3993	4064	1446	634
Cover sprays	3899	3541	2432	1346	419

Table 12c. Estimates of total loads of fruit per tree - persimmon in Mardan, 1998.

Date:-	1	2	3	4
BAT	1063	863	763	886
Unsprayed	1256	942	693	1075

Table 12d. Estimates of total loads of fruit per tree - ber in D.I.Khan, 1998.

Date:-	1	2
BAT	2037	300
Unsprayed	1440	244

Table 12e. Estimates of total loads of fruit per tree - lufa in R.Y.Khan, 1998.

Farm	Date:-	1	2
RYK1	BAT	87	63
	Unsprayed	34	60
RYK2	BAT	78	47
	Unsprayed	65	51

Again, the results indicate a higher yield by the protected plots, although this appears less than when percentage infestation alone is compared. It may be that a more reliable estimate of the protection value of a control method is given by the percentage infestation values, as fruit production varies too extremely under natural conditions.

Ideally, these figures would approximate those for the records of harvests discussed in section 1, but unfortunately there are only two reliable comparable data points (RYK1) so further exploration of this relationship will have to wait.

7. Conclusion

All the results above indicate that bait sprays are (a) effective in controlling fruit flies on guava, where they are traditionally a most serious pest and (b) as good as if not better than cover sprays, to which they are decidedly preferable for reasons of cost, safety and environmental contamination. In all the cases where bait sprays did not provide significant protection, this may be attributed to starting too late (due to inevitable delays establishing trials in early 1998), sampling problems mainly due to communications and transport difficulties (inevitable in a project spread over such a huge area) or the loss of plots to other problems such as drought. Even in some of these cases there were encouraging signs (as trap catches in R.Y. Khan) of the effect of baits on fly populations.

Many questions remain to be answered, particularly the more mathematically detailed questions of distributions. It is hoped that more data, and of greater completeness, will be gathered in 1999: when this is gathered, the entire data set, including those above, will be analysed in an integrated way. The above therefore represents only a stage on the way to the presentation of a comprehensive analysis.

INFORMAL FARMER-MANAGED TRIALS OF BAIT SPRAYS

Figure 10. Meeting with farmers at a mango orchard outside Rahim Yar Khan in November, 1998. The four figures in western dress are, from the left, Mr Imtiaz Ahmad of the Rahim Yar Khan Agricultural Extension Service, and the authors Mahmood, Stonehouse and Huggett.

The project is accompanying the full-scale quantified formal trials of the “main study” with a series of informal, only-broadly-quantified farmer-managed trials. These involved the assessment of bait sprays only, by the basic process of giving farmers material on an experimental basis, with training on how to use it, and then recording their reports of their results, opinions and recommendations in their own terms (it was stressed that the preparation was experimental in nature, and that its ready availability for the foreseeable future could not be relied on; farmers participating are those with managerial attitudes and aware of the vicissitudes of technology under development). Only two of these have reported results so far, as reported below, but the programme is expanding considerably in 1999 and it is hoped to obtain some results from these which:-

1 - acquire additional information about the effectiveness of controls for little extra cost, as a way of backing up and corroborating the more exhaustive and exhausting records of the main study.

2 - acquire more farmer-relevant information by focussing on farmers’ perceptions of the advantages and disadvantages of the technology, thus allowing refinement of the technology to meet real needs.

The results obtained so far have been as follows.

1 - BAT of guava tried by a member of the research staff and regional project manager on three guava trees in his garden. He has 3 guava trees in his own back yard and has taken to squirting them, on an experimental basis, every Sunday, around the trunk and foliage with bait spray mixture in a hand-held plastic domestic

garden sprayer. According to him this is effective in protecting his guavas, which last year were virtually destroyed by flies, as were those of his neighbours, whereas now he has so many he can give them to the neighbours, whose own guavas remain very heavily infested. This result may be startling in its implications, as it has long been assumed that bait sprays have some scale effect, and that areas as small as this may not be defensibly by bait sprays, particularly with unprotected hosts abundant nearby, as arriving oviposited females may have fed on protein elsewhere and thus not be attracted to the bait. This implies that, on the contrary, arriving females may be attracted to protein, if it is there, before attacking fruit anyway (an approach which makes some evolutionary sense if protein is scarce in the environment - such an abundant and attractive source as protein hydrolysate may be to be exploited by feeding as a priority). This is encouraging, and may be further looked at.

The following quantitative approximations were obtained for this trial:

- Loss in typical previous year - 70-80%
- Loss with protection, 1998 - 10-20%
- Loss in nearby plots (neighbours' gardens) - >50%
- Loss in nearby plots, previous years - >50% (i.e. "No reduction" in 1998)

The losses in neighbours' trees were only over the fence; a pair of traps also placed in the garden for interest are considered unlikely to be responsible for the low level of attack as presumed also to protect near-neighbouring trees.

2 - BAT of ber in Khanpur, Punjab (described in more detail elsewhere under *Field visit reports, March 1999, Farm 2, Khanpur* in Appendix 23). This farmer tried a single BAT spray instead of one of three cover insecticide sprays to protect ber fruit from fruit flies; the first of his conventional three had been ineffective (it may have washed off in rain) and the bait spray was so successful that the other cover sprays were abandoned. The orchard gave the distinct impression of a heavy fruit yield, as far as damage to branches by the weight, as shown in the photograph on the front cover, and the harvest team were evidently delighted. No disadvantages were reported. Quantified estimates were almost impossible to elicit from the farmers (not least because so busy in gathering the harvest) but an estimate that production was approximately double the typical one (*i.e.* with the three cover sprays) was obtained.

V. CONTRIBUTION OF OUTPUTS

The findings presented above represent the majority of the research findings at the time of writing in July 1999. It must be stressed that, although UK funding for technical assistance has ceased, the project is still very much in progress, with Pakistani partners engaged in substantial research work for 1999. Results from the 1999 field season are anticipated to be at least as significant as those from 1998, and Imperial College staff have undertaken to provide from a distance whatever assistance is necessary for the full analysis, evaluation and reporting of the results. Furthermore the work of the student researchers, though started and producing useful results in the validation of techniques last year, is expected to produce major results in the 1999 field season. The 1999 season will also see the first trials of Male Annihilation Technique and an expansion of the programme of informal, farmer-managed trials. The next final report will substantially expand on the information presented here. Appendix 5 outlines the strategy for Male Annihilation Technique research, Appendices 6 and 7 the student work on developing cheap home-made protein baits, Appendix 8 the student work on the relationship between attack and damage, and Appendix 9 the student work on soaked killer blocks for MAT.

As mentioned above, a concern was the possibility that the uptake of improved fly control methods by wealthier farmers may expand fruit production, which would depress prices and so reduce the incomes of poorer farmers unable to invest in control improvements themselves. To investigate this risk, a small economic study was undertaken to analyse the relationships between volumes marketed and prices, for mangoes, guavas, melons and other fruits, and the results are given in Appendix 10. They are rather inconclusive, but provide little evidence of a strong likelihood of the expansion of production leading to serious reductions in prices.

Other activities than research were an integral part of this project. Information about the project's activities, findings and recommendations have been distributed to participating extension services, and to officials at senior levels in the Pakistan Agricultural Research Council. A two-day training workshop was carried out at CABI Biosciences in March 1999 and was well received; the attendance, session schedule and some of the talks presented are given in Appendix 11. Researchers and students were provided with detailed teaching in the methods and information needed for their research. Participants were provided by the project with equipment needed for the assessment of fruit fly infestations in the field (listed in Appendix 12) as well as training, rehearsal and practice in techniques. Copies of key fruit fly literature references (listed in Appendix 13) were deposited in the library at CABI Biosciences in Rawalpindi to form a small fruit fly document centre.

REFERENCES

Bersten, David (1997). Phloxine B - A photoactive insecticide. *Pesticide Outlook*, October, 20-23.

Latif, Abdul, Marwat, Nawab Khan, & Hussain, Naseer (1987). Suppression of population and infestation of *Dacus* spp. fruit flies through the use of protein hydrolysate. *Sarhad Journal of Agriculture* **3**: 509-13.

Marwat, Nawab Khan, Hussain, Naseer, & Khan, Ayub (1992). Suppression of *Dacus* spp. by male annihilation in guava orchard. *Pakistan Journal of Zoology* **24**, 82-4.

Qureshi, Z.A., Bughio, A.R., & Siddiqui, Q.H. (1981). Population suppression of fruit fly, *Dacus zonatus* (Saund.) (Dipt., Tephritidae) by male annihilation technique and its impact on fruit infestation. *Zeitschrift für Angewandte Entomologie* **91**, 521-4.

Siegel, Sidney, & Castellan, John R. (1988). *Nonparametric Statistics for the Behavioural Sciences.* New York: McGraw-Hill. 347pp.

Sokal, Robert R., & Rohlf, F. James (1981). *Biometry.* New York: W.H. Freeman. Second edition. 859pp.

Stonehouse, John M. (1997). *Fruit Flies in Pakistan: Needs Assessment for a Control Research Project.* Report for Natural Resources International. 24pp.

Stonehouse, John M., Mumford, John D., & Mustafa, Ghulam (1998). Economic losses to Tephritid fruit flies (Diptera: Tephritidae) in Pakistan. *Crop Protection* **17**: 159-64.

Stride, Barry (1997). *Six Month Technical Report - Plant Protection Activities 01 May - 31 October, 1997*. UN FAO Project: Integrated Crop and Food Production in Afghanistan. Unpublished. AFG/96/004