

VG408

**Quantitative evaluation of the systems
used to meet New Zealand import
requirements for cucurbit crops**

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QDPI & New Zealand Plant Protection
Centre - Lynfield**



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VG408

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FINAL REPORT

HRDC PROJECT VG 408

Quantitative evaluation of the systems used to meet New Zealand import requirements for cucurbit crops.

INTRODUCTION

The project, led by Dr R Drew was planned in close collaboration with scientists from the New Zealand Plant Protection Centre - Lynfield. All research activities were carried out by DPI staff in the Market Access Technology Group of the Plant Protection Unit in Brisbane. Assistance with field trials was provided by staff at DPI Research Stations at Redlands and Bundaberg.

The project commenced in July 1994 and experimental work was completed in December 1994. A detailed technical report of research activities and statistical analysis of results was produced by the NZ investigators in collaboration with Dr Drew in February 1995. This report entitled "Development of Pest Risk Management Options based on a Whole Systems Approach. 1. Efficacy of Quality Production Systems" was not submitted to HRDC at that time. A Final Report on Project V6408 had not been submitted when Dr Drew left DPI in January 1997.

Dr Annice Lloyd who was one of the investigating team in this project and who is now Senior Research Scientist (Fruit Fly Control) has prepared the Industry and Technical Summaries to accompany the NZ prepared document in this Final Report.

FUNDING

Project VG408 was funded by Queensland Fruit & Vegetable Growers.

HRDC PROJECT VG408 - Quantitative evaluation of the systems used to meet New Zealand import requirements for cucurbit crops.

INDUSTRY SUMMARY

Background:

The export of Australian horticultural produce has, in the past depended on post-harvest treatments designed to kill in excess of 99.9968% of pests (Probit 9). Such treatments are frequently in excess of what would be required to prevent the introduction of a pest species into an importing country. Physical (heat or cold) post-harvest treatments are now the preferred option to chemical treatments. This means that more emphasis is being placed on developing minimal post-harvest treatments which will meet the quarantine requirements of importing countries whilst still maintaining acceptable quality.

This approach to meeting quarantine restrictions has increased the need for pre-harvest pest risk management options. Such a whole systems approach includes consideration of quality production systems, the area freedom concept and host status as well as post-harvest disinfestation treatments as means by which quarantine specifications may be met. To use a quality production system as a risk management option, data must be obtained on the infestation level of the export product at selected key points along the production-to-export pathway.

Research:

This project has succeeded in obtaining the above data for zucchinis grown commercially in Queensland for export to NZ. The fruit fly species which attacks cucurbit crops, including zucchinis in Queensland is *Bactrocera cucumis*. This study evaluated the ability of a quality production system to reduce infestation levels of *B. cucumis* at two selected key points in the commercial production pathway. The points chosen were at harvest and at the end of the packing and transport process i.e. when the zucchinis had arrived at the departure point for export. This enabled the efficacy of field control methods, and of packing shed quality control and transport security in reducing fruit fly infestation levels to be quantified.

This research involved sampling approximately 33,000 zucchinis over a period of 13 weeks during peak production. Results showed that there was a 2.5% infestation level in unsprayed control plots. In trial plots grown under normal commercial spray regimes, no infestation was detected in a total of 15,346 export fruit at harvest or in a total of 15,575 export fruit after packing and transport.

Outcomes:

These results demonstrated that the pre-harvest fruit fly control measures and post harvest quality control procedures were capable of reducing field infestation below a level detectable with repeated high-intensity sampling. Statistical analysis of the results showed that a post harvest treatment efficacy equivalent to probit 7.05 would be required to meet NZ quarantine requirements. Such a treatment would be much lower than the traditional probit 9 requirement thus demonstrating the value of pest risk analyses in a whole systems approach. On the basis of these results, a relatively mild physical treatment such as hot water dipping could be developed to meet the NZ requirements as an alternative to a chemical post-harvest treatment. This would have the advantage of minimizing chemical application but at the same time ensuring export quality produce.

HRDC PROJECT VG408 - Quantitative evaluation of the systems used to meet New Zealand import requirements for cucurbit crops.

TECHNICAL SUMMARY

Aim: To quantitatively evaluate fruit fly infestation levels in zucchinis grown in Queensland for export to New Zealand.

Methods:

This project was planned in collaboration with scientists from NZ MAF Quality Management. Research activities were carried out by DPI staff in the Market Access Technology Group, Plant Protection Unit, Brisbane. A pilot trial was undertaken on zucchinis grown at Redlands Horticultural Research Station near Brisbane. The two main trials were carried out using zucchinis grown for export on a commercial farm at Bundaberg, Queensland. An unsprayed control plot was grown at the DPI Bundaberg Research Station at the same time as the main trials.

In the trial plots, the crop was grown with normal commercial applications of pest and disease control treatments. Fruit was sampled over 2 periods (Aug - Sept 1994 and Oct-Nov 1994) during the main export production season. All field sampling was done according to a pre-determined, statistically designed procedure. Fruit of export size only (12-18 cm) were picked and trial plots and control plots were sampled on the same day. Samples were taken at two points in the production system, at harvest and at packing for export, i.e. after fruit had passed through normal quality control procedures and had been transported to Brisbane. All fruit were held in controlled temperature conditions at the DPI laboratory in Brisbane for 7 days before being individually examined for fruit fly infestation.

Results:

In unsprayed control plots, 49 fruit from a total of 1956 were found to be infested i.e. infestation level of 2.5%. No fruit fly infestation was found in 15,346 export field harvested fruit or in an additional 15,575 fruit which had been through the normal commercial quality control procedures and had been transported to Brisbane.

Outcome:

The results of this project demonstrated that a quality production system based on properly applied pre-harvest control measures and post harvest quality control checks can reduce fruit fly infestation to a very low level. Statistical analysis of the results showed that a post-harvest treatment of efficacy 7.05 instead of the traditional probit 9 would meet NZ quarantine requirements for the export of Queensland grown zucchinis.

Recommendations

- Research should be undertaken to develop a non-chemical post-harvest treatment of probit 7.05 (eg hot water dipping) for zucchinis to be exported to NZ.
- This project has demonstrated the value of a whole systems approach in pest risk management of fruit flies. Research to extend this concept to other fruit fly host commodities should be undertaken.

Development of Pest Risk Management Options based on a Whole Systems Approach

1. Efficacy of Quality Production Systems

Development of Pest Risk Management Options based on a Whole Systems Approach

1. Efficacy of Quality Production Systems

INTRODUCTION

The Whole Systems Approach

Historically, most countries have imposed entry conditions on the importation of plants and plant products. These conditions have rarely been developed in the context of risk management as now understood by FAO (Anon 1993). The most common method of preventing pests becoming established in new areas as a result of trade, has been to require postharvest treatments designed to kill in excess of 99.9968% of pests ("probit 9"). The efficacy of such treatments were in most cases greatly in excess of that required to prevent pest establishment. The result was unjustifiably harsh quarantine requirements causing unnecessary expenditure to the exporting country. It is also clear that the almost universal acceptance of fumigation as the preferred risk management option stifled research into the development of other methods.

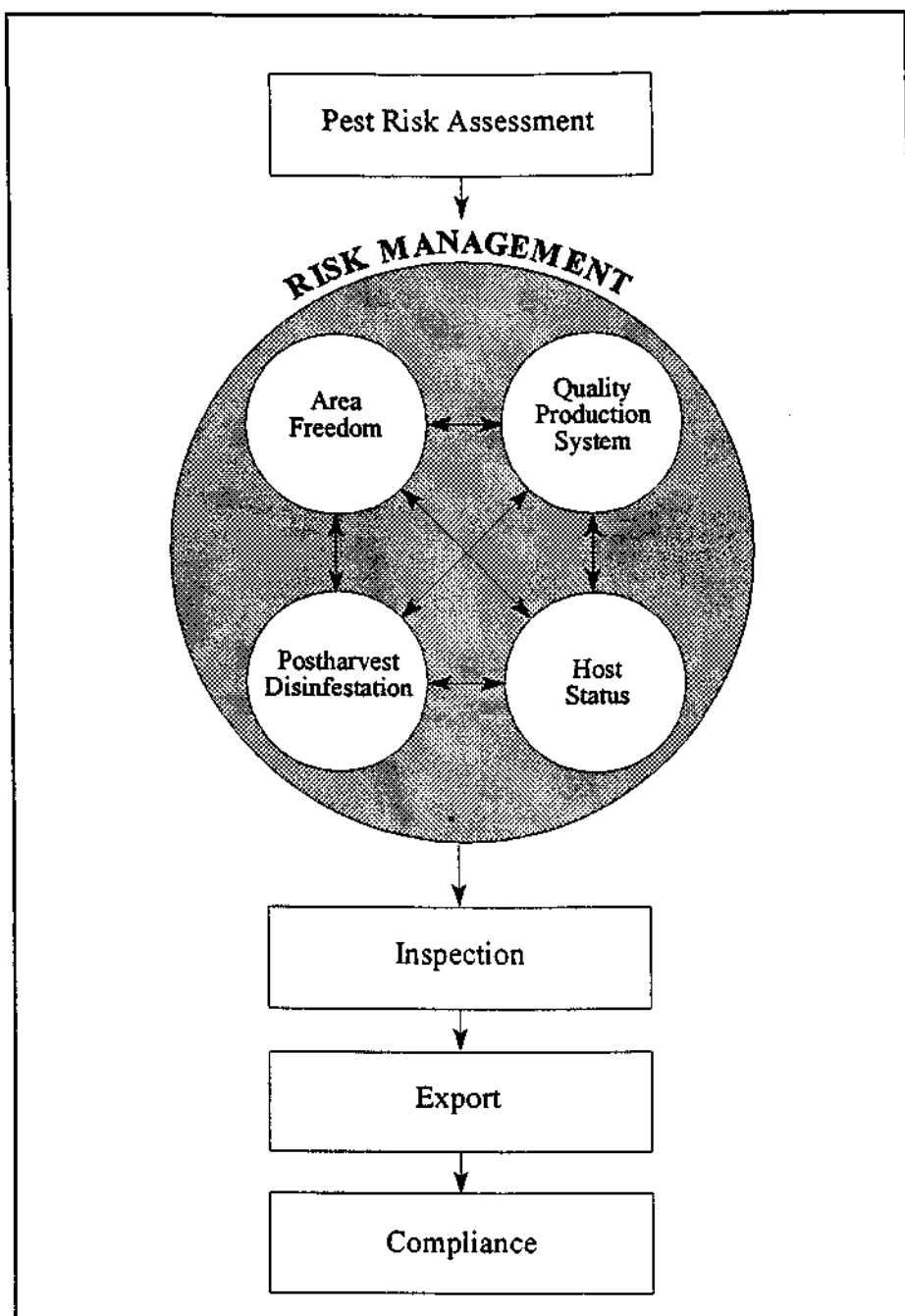
With reference to pest risk management the onus has always been on exporters to satisfy the requirements of the quarantine authorities of an importing country. These authorities have a very clear goal ... to prevent the introduction of harmful, exotic pests into their countries. At the same time they realise they have a role in the facilitation of trade. This means that a level of risk has to be accepted and managed. Thus, in this context, quarantine authorities are faced with the development of risk management options which must satisfy two often opposing ideals. The risk of introducing pests must be minimised while allowing relatively unhindered trade in fresh produce.

If the risk management options developed for trade in fresh produce are to be fully justified they must be based on impeccably sound, mutually agreed scientific research. Research requirements to support the exportation of fruit fly host material have been suggested by Baker et al. (1994). It is clearly the responsibility of the exporting country to provide such research data. The importing country, however, has an important role. It is essential that clear, justifiable tolerances are declared for all pests of concern. These may be calculated using probability models for pest risk assessment, or assigned by subjective decisions, but unless they are stated and agreed to by the importing and exporting authorities, there is no real basis for research. Furthermore any research carried out for specific pest risk management reasons should follow mutually agreed Standards.

The focus for developing entry conditions should be on a whole system approach based on pest risk analysis [PRA] (Anon 1993, Baker et al. 1993). This approach will lead to more emphasis on the use of area freedom, host status and quality production systems. The efficacy of these factors can be calculated to ensure that tolerances imposed by importing countries are not exceeded, thereby reducing reliance on postharvest disinfestation treatments. The use of the whole system approach is seen as compatible with world-wide trends towards more environmentally friendly pest management, and sustainable agriculture.

The whole systems approach to pest risk management options includes consideration of quality production systems, the area freedom concept and host status, as well as postharvest disinfestation treatments. This is summarised in Figure 1.

Figure 1: The whole system of risk management options



Risk management options can be used alone or in combination to meet the specification. Options can reduce the infestation level of the product, the number of individuals present in a product, the survival of individuals in the product, or a combination of these effects. For any combination of risk management options, data on infestation level, numbers of pests, or pest survival are required. Use of a quality production system as risk management requires data on the infestation level of the export product. The risk management model is then used to determine whether the infestation level meets the specification.

OBJECTIVE

The objective of this study was to show quantitatively how quality production systems can be used as risk management to meet the specifications for fresh produce imported into New Zealand. This work included the development of a general risk management model based on the pest risk assessment procedure of Baker et al. (1993), and a research project establishing protocols for measurement of the components of a quality production system. The research project focused on the ability of production systems to reduce infestation levels of *Bactrocera cucumis* in Queensland zucchini crops destined for export to New Zealand. Measurement of infestation levels at selected key points along the export pathway (see Figure 2, page 5) were made by staff of Queensland Department of Primary Industries (QDPI). These points coincided with the harvesting of the crop, with the end of the packing procedure and with the arrival of fruit at the airport in Brisbane. The production system studied was able to justify a reduction in postharvest treatment strength from 0.0023 (probit 7.83) to 0.02 (probit 7.05).

General Model for Risk Management

The risk management model is based on a specification, in terms of percentage of items infested, calculated using the PRA procedure of Baker et al. (1993) with some modifications.

The specification (M_s) is calculated as $100\% \times M$, where

$$M = \frac{1 - \sqrt[Nm]{1 - r}}{\Phi} \quad (1)$$

(Baker et al. 1993).

In this case, Φ is the probability of an establishment from an infested item. This is calculated as the probability that an infested item is disposed of where and when conditions (such as climate or host availability) are suitable for development, multiplied by the probability that enough individuals survive to form a mating pair. The probability of encountering suitable conditions is calculated as the product of survival probabilities $C_4 \times C_7 \times C_8$ (see Baker et al. 1993). If sex ratios are equal and the number of individuals present follows a Poisson distribution with mean λ , the probability of a mating pair surviving is calculated as

$$Pr(\text{mating pair}) = 1 + e^{-\lambda} - 2e^{-\lambda/2} \quad (2)$$

(Landolt et al. 1984, Baker et al. 1990).

In this case, λ is expressed as $\mu\phi\varphi$, where μ is the number of individuals present in the item (such as the average number of eggs in an infested fruit), ϕ is the probability of an individual surviving factors such as natural mortality or disposal site mortality, and φ is the probability of an individual surviving risk management. The value of ϕ is calculated as the product of $C_2 \times C_3 \times C_5 \times C_6$ (see Baker et al. 1993), all factors which influence individual survival.

Thus, the general risk management model becomes

$$M = \frac{1 - \sqrt[Nm]{1 - r}}{\frac{(1 + e^{-\mu\phi\varphi} - 2e^{-\mu\phi\varphi/2})}{1 - e^{-\mu}} \times C_4 \times C_7 \times C_8} \quad (3)$$

The term $1 - e^{-\mu}$ in the denominator is a correction for the probability of 0 individuals present, which is included in the Poisson probability but not actually possible given the requirement that the fruit be infested. This is different from the probability of 0 individuals surviving, and so μ is not multiplied by $\phi\varphi$ in this term.

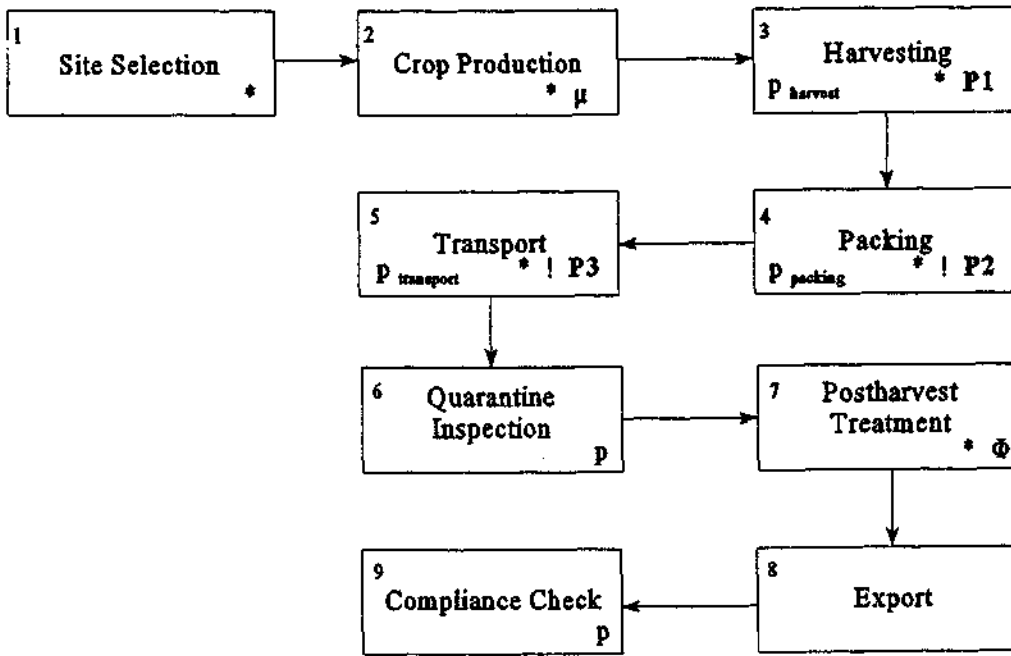
It is possible that the number of individuals per infested fruit will not follow a Poisson distribution, but a clumped distribution such as the Neyman Type A or negative binomial. In this case, the calculation for the probability of a mating pair surviving will differ from Equations 2-3. However, in most cases the Poisson distribution results in a slightly greater chance of a mating pair surviving than does a clumped distribution. Thus, Equation 3 gives a reasonable, slightly conservative, approximation for M .

If M is less than the infestation level detectable by visual inspection (generally 0.005), risk management will be required. Risk management must either demonstrate that the infestation level, p , of the exported product is $\leq M$, or provide new values for μ , ϕ , and/or φ which ensure that $M \geq p$, with 95% confidence.

COMPONENTS OF QUALITY PRODUCTION SYSTEMS

The steps involved in a typical production - export system are summarized in Figure 2. Each step has the potential to influence pest infestation levels, mostly by reducing them.

Figure 2: Steps in the production - to - export process



- * = critical point where infestation level may be decreased
- μ = number of fruit flies per infested fruit
- P = efficacy of system
- Φ = efficacy of treatment
- p = infestation level
- ! = critical point where infestation level may be increased

This report concentrates solely on quality production systems (steps 1-5 in Figure 2) and the role they play in reducing pest infestation levels.

The efficacy of a production system is analogous to that of a postharvest treatment. For a postharvest treatment, efficacy is the proportion of pests remaining after treatment. For a production system, efficacy is the proportion of infested fruit remaining in the end product at the time it is presented for inspection prior to export. The overall production system efficacy can be broken down into various components for research and calculation purposes. Each component represents an infestation level at one step of the production process.

Research Components

The following components can be incorporated into the overall production efficacy.

- **p_{control} = proportion of harvested, untreated produce that is infested by pests**

Measurement of this factor indicates the natural level of pest infestation in the absence of pest management.

- **p_{harvest} = proportion of harvested, commercially grown produce that is infested by pests**

Measurement of this factor indicates the efficacy of actions taken to minimize pest infestation during production.

- **p_{packing} = proportion of produce in packed, commercially grown export produce that is infested by pests**

Measurement of this factor indicates the efficacy of actions taken to minimize pest infestation in the packhouse.

- **$p_{\text{transport}}$ = proportion of produce in packed, commercially grown export produce arriving at the point of export that is infested by pests**

Measurement of this factor indicates the efficacy of actions taken to minimize pest infestation during transport of the packed product to the point of export.

The efficacy of the pre-harvest control measure (site selection, crop spraying or other pest management practices) is calculated as the infestation level of export quality produce at harvest time, p_{harvest} divided by the infestation level in control produce, p_{control} . The efficacy of packhouse practices (grading, sorting, packing) is calculated as the infestation level of packed produce, p_{packing} divided by p_{harvest} . Transport to the point of export is another step for which efficacy can be calculated. During transport, infestation level could be increased (produce inadequately protected from pests) or decreased (pests suffering mortality due to transit conditions). Transport efficacy is calculated as the infestation level of the produce arriving at the point of export, $p_{\text{transport}}$ divided by p_{packing} . In many cases produce is protected during transport and pest mortality is likely to be negligible so that transport efficacy = 1 (i.e., $p_{\text{transport}} = p_{\text{packing}}$).

Therefore, the various infestation levels for the production system are:

$$P_1 = p_{\text{harvest}}/p_{\text{control}}$$

$$P_2 = p_{\text{packing}}/p_{\text{harvest}}$$

$$P_3 = p_{\text{transport}}/p_{\text{packing}}$$

$$\text{and overall } P = P_1 \times P_2 \times P_3 = p_{\text{transport}}/p_{\text{control}}$$

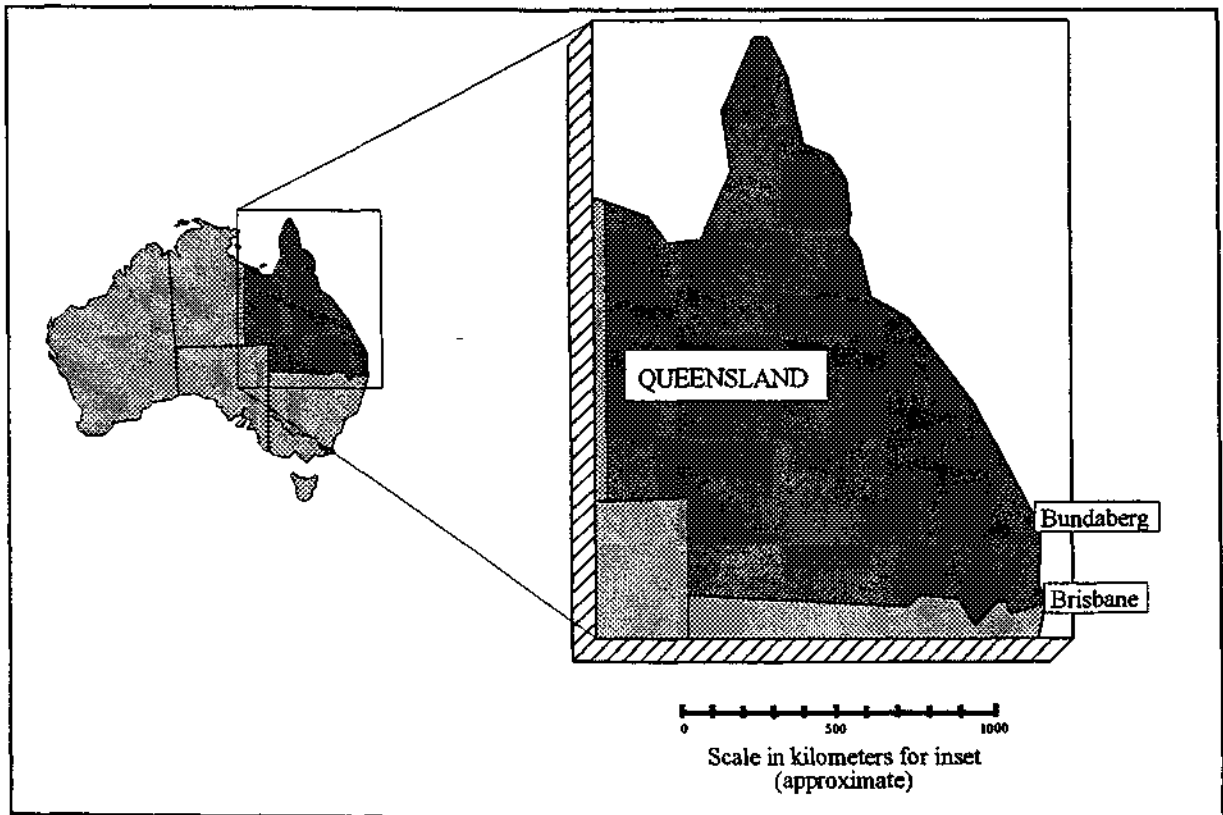
The endpoint infestation level, $p_{\text{transport}}$ is then compared with M in the general model.

MEASUREMENT OF PRODUCTION SYSTEM COMPONENTS

Field Trials

A pilot trial on zucchini was carried out near Brisbane at the Redlands Horticulture Research Station, Queensland. The main trials used zucchini destined for export to New Zealand grown at Trevor Farmlands in Bundaberg, Queensland (see map, Figure 3). Control plots for the main trials were grown by Queensland Department of Primary Industries (QDPI) staff at the Bundaberg Research Station. All trials were supervised by Dr R.A.I. Drew of QDPI.

Figure 3: Location of research sites



Methods

Redlands Pilot Trial

The pilot trial at Redlands consisted of 350 plants, in 5 rows 70m long, planted on 20 December 1993. The study plot was treated with fungicides, but no insecticides.

Sampling began on 12 January 1994. At weekly intervals, all fruit were removed from the plants and held in the QDPI rearing facility at Indooroopilly, Brisbane, for assessment of infestation. In addition, twenty plants were selected at random, and the number and size of fruit produced weekly by each plant was monitored. Sampling continued until 23 February (7 weeks), by which time a large proportion of plants had succumbed to fungal attacks.

Flies were trapped using orange-ammonia or protein bait lures. One trap of each type was located in the zucchini plot, and additional traps were sited each side of the plot in tall grass buffer zones. Traps were first baited on 21 January 1994, and cleared at weekly intervals until 23 February (5 samples). All *Bactrocera cucumis* in the traps were preserved in alcohol and later sorted by sex and reproductive maturity.

Main Trials

Two trials were carried out, the first with picking dates from 2 August (10 August for controls) - 21 September 1994, for 8 (7) weekly samples, and the second from 10 October - 14 November 1994, for 6 weekly samples. The second trial was shorter because the plants finished producing fruit earlier as the season progressed. For each trial, control plot plants were grown in the same manner as plants at the export plots, but were not treated with insecticide. Samples were taken from control plots, export plots, and from packed export fruit transported to Brisbane, to measure p_{control} , p_{harvest} and $p_{\text{transport}}$ (equal to p_{packing} in this system). Research protocols (work plans) for both the control and export plots, and for fly trapping are given in Appendix 1. Differences between procedures in Trials 1 and 2 are noted. Copies of forms used to record data are included in Appendix 2.

Results: Redlands Pilot Trial

Trap Effectiveness

The orange-ammonia traps consistently caught higher numbers of flies of both sexes each week than did the protein bait traps (Figure 4). The sex ratio of flies caught was similar for both types of traps, although it varied by date (Figure 5), with males making up approximately 12 - 42% of the flies caught.

Figure 4: Total number of flies trapped

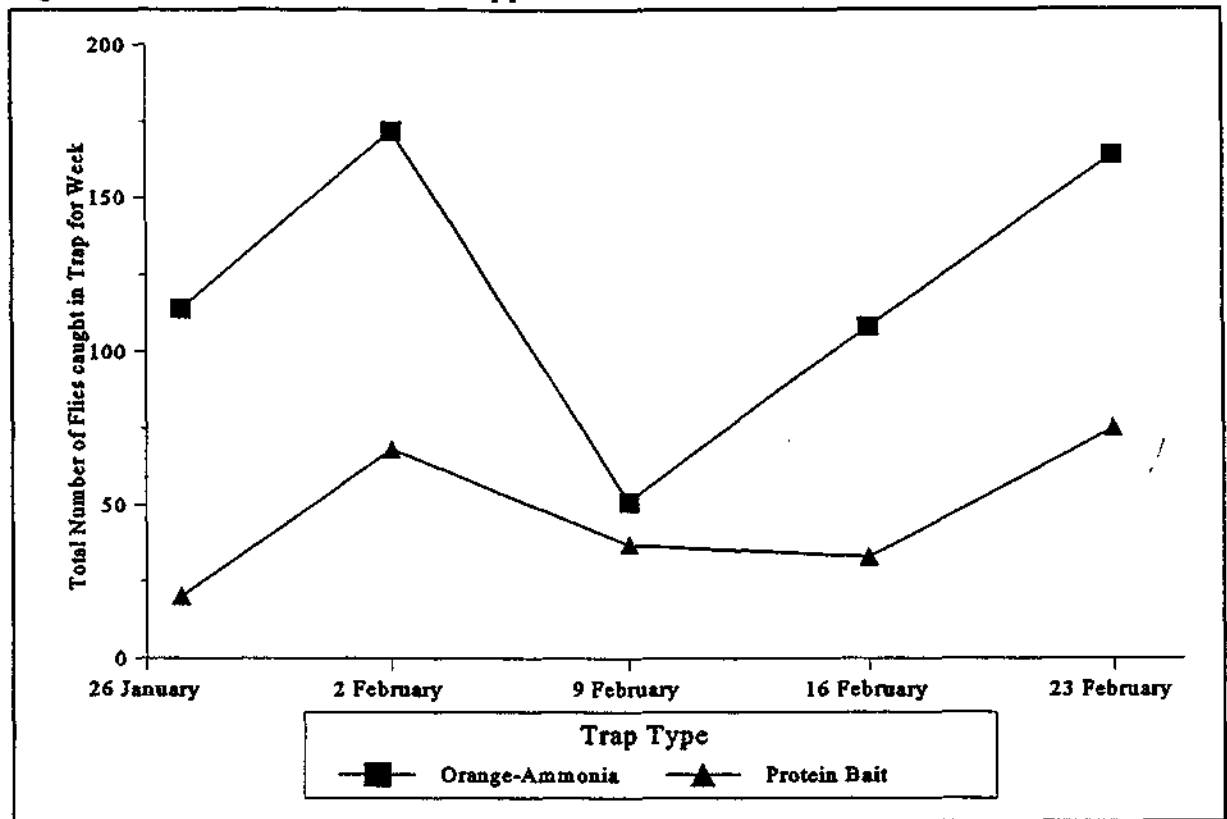
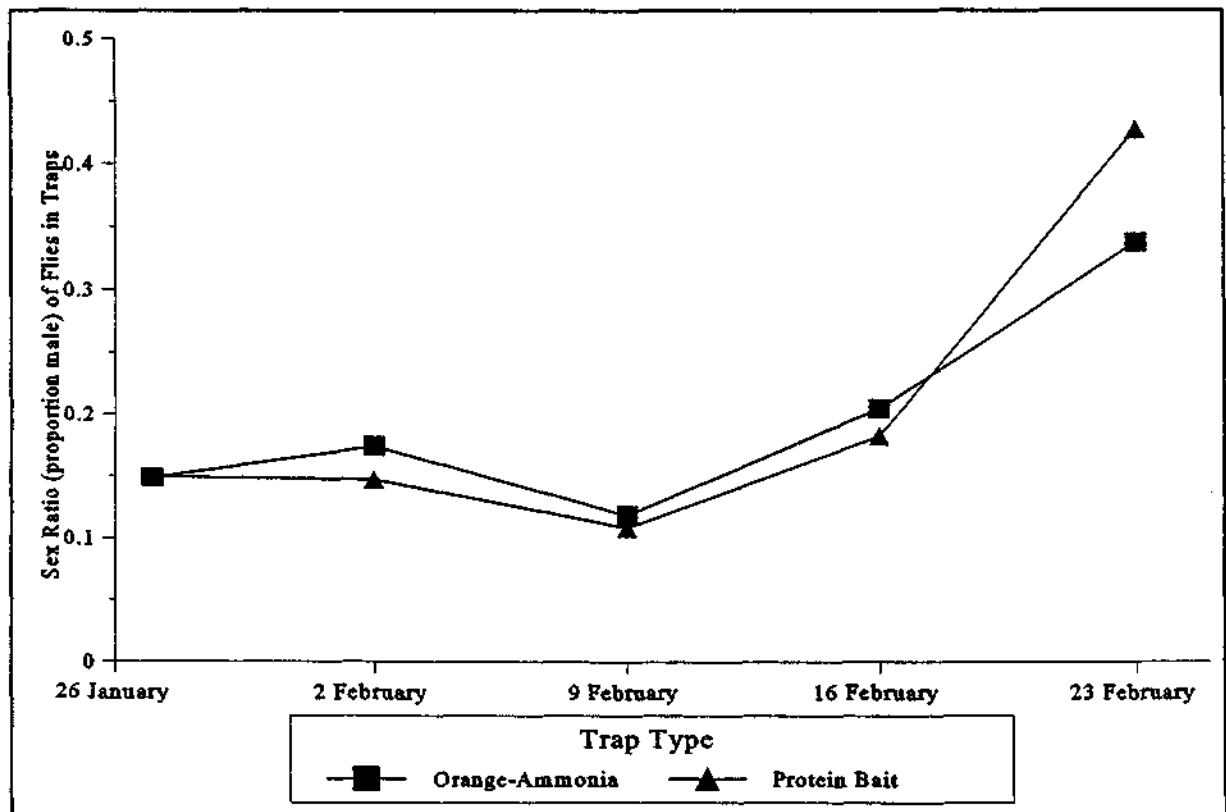


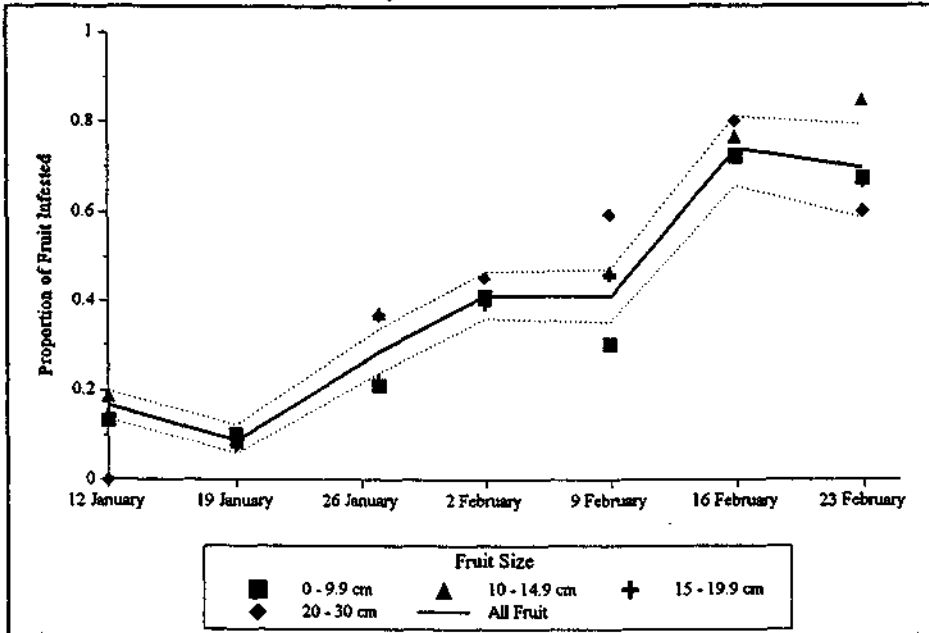
Figure 5: Sex ratio of flies in traps (proportion males)



Infestation Level

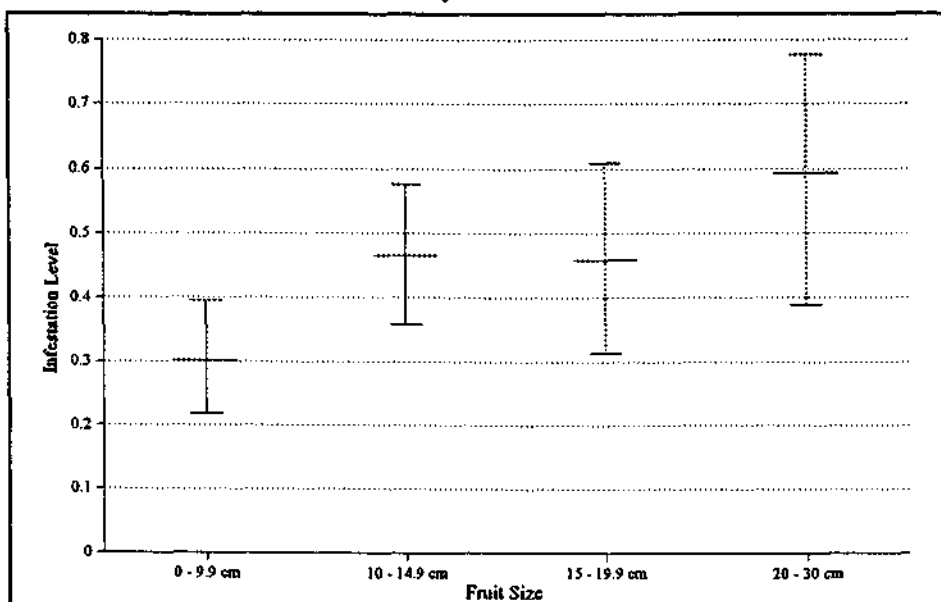
As shown in Figure 6, infestation levels of all size classes increased over the duration of the trial. Dashed lines show the 95% confidence limits for proportion of total fruit infested.

Figure 6: Infestation level of fruit by date and size



Although larger fruit tended to have higher infestation levels, pairwise differences were generally not significant between groups at the 0.05 level, except between the largest and smallest size categories. Figure 7 shows the infestation levels for fruit in each size class on 9 February, with 95% confidence limits. Chi-square analysis of proportions reveals an overall significant difference among groups ($\chi^2 = 10.835$, $p = 0.0127$); however, this is due to differences between the 0-9.9 cm group and the 20-30 cm group. The 10-14.9 and 15-19.9 cm groups show considerable overlap with both the 0-9.9 and 20-30 cm groups.

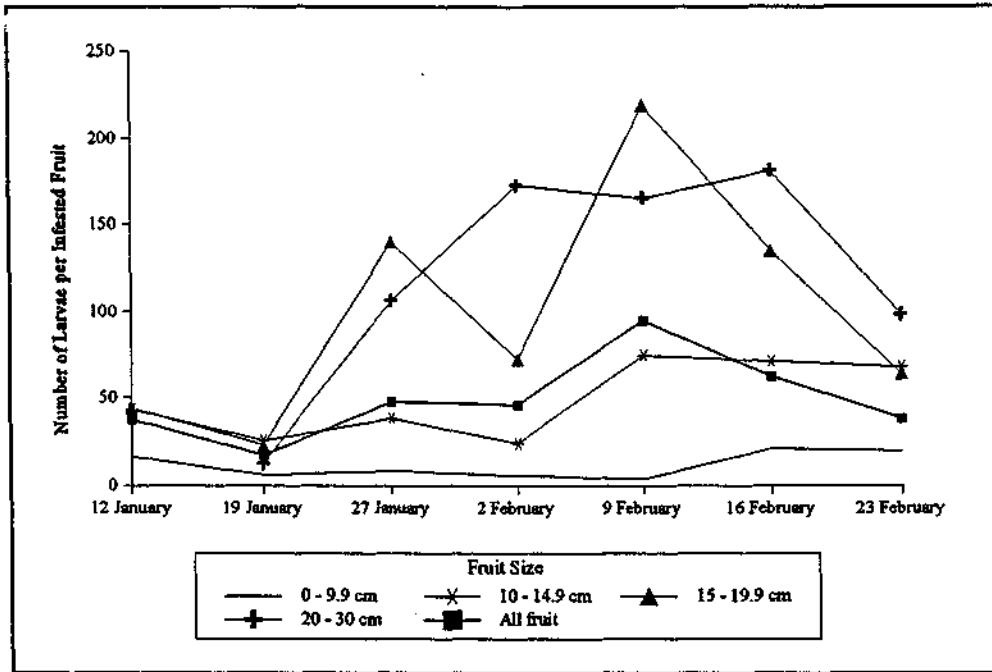
Figure 7: Infestation levels on 9 February for fruit in each size class



Larvae per Infested Fruit

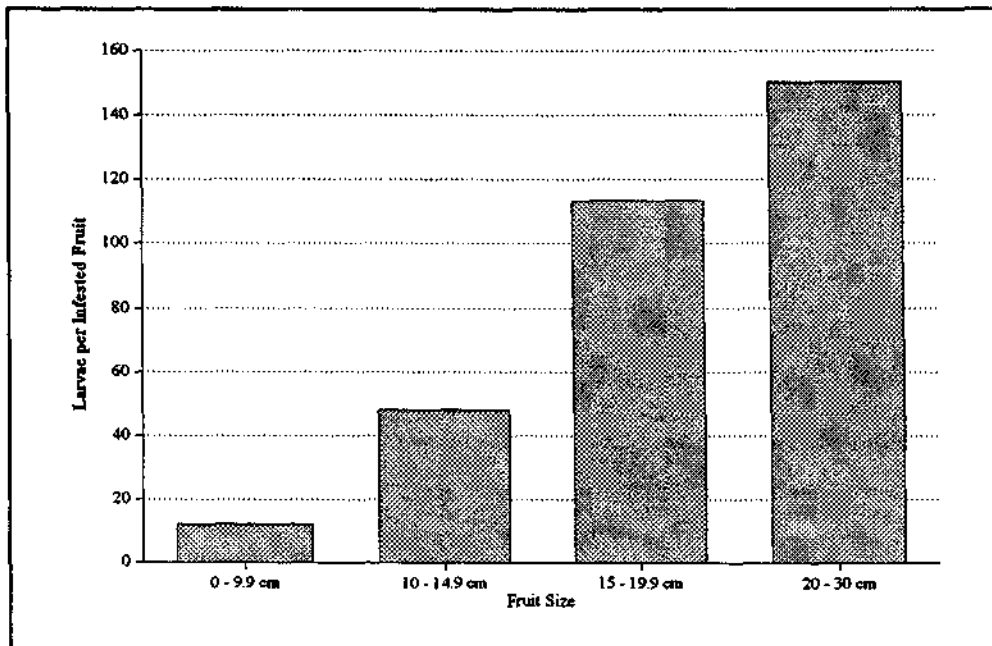
The number of larvae per infested fruit increased during the first part of the trial for the larger size categories, decreasing in the last two weeks. The 0-9.9 cm fruit remained relatively constant throughout the trial (Figure 8).

Figure 8: Number of larvae per infested fruit by size class and date



Averaging across dates for each size class shows that the mean number of larvae per fruit increased steadily with increasing fruit size (Figure 9). This is expected, as larger fruit remain in the field longer, and are more likely to have multiple infestations.

Figure 9: Mean number of larvae per infested fruit by fruit size



The number of larvae in individual fruit was not counted, as infested fruit in the same container rapidly broke down and ran together, so that it was impossible to tell from which fruit larvae came. Thus, no direct measure of variability in numbers of larvae per fruit for a particular date and size category was obtainable.

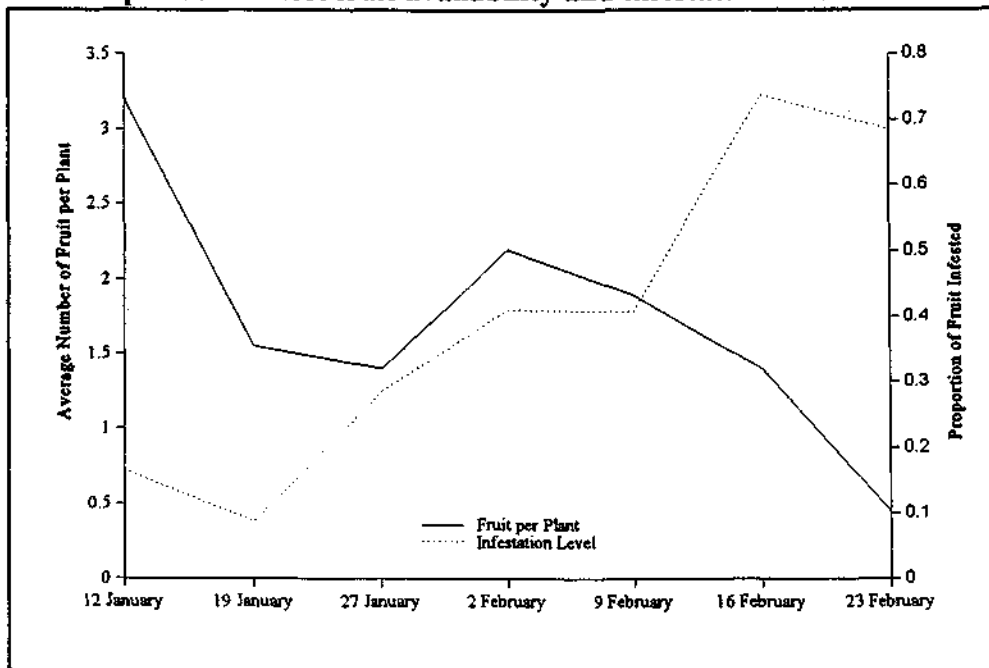
Seasonality

Several factors correlated with time. The natural logarithm (ln) of male flies in the traps showed a strong positive correlation with date ($r = 0.40$ for orange ammonia, $r = 0.71$ for protein bait). Total numbers of flies showed weaker correlations ($r = 0.17$ for orange ammonia and $r = 0.54$ for protein bait). When infestation levels were transformed to logits, where $\text{logit}(p) = \log[p/(1-p)]$, proportion of export fruit infested was highly correlated with date ($r = 0.93$) and also with ln of larvae per infested fruit ($r = 0.71$). This shows that in general, as the growing season progresses and fly populations increase, infestation levels and numbers of larvae per infested fruit also tend to increase.

Numbers of larvae per infested fruit (both export size and total fruit) showed negative correlations with numbers of flies caught in traps. Infestation level, however, showed weak to moderate positive correlations with numbers of flies trapped.

Host fruit availability also generally declined over time, although a second flush of fruit occurred midway through the trial on 2 February. Figure 10 shows a comparison of average number of fruit (all sizes) per plant and infestation level.

Figure 10: Comparison of host fruit availability and infestation level



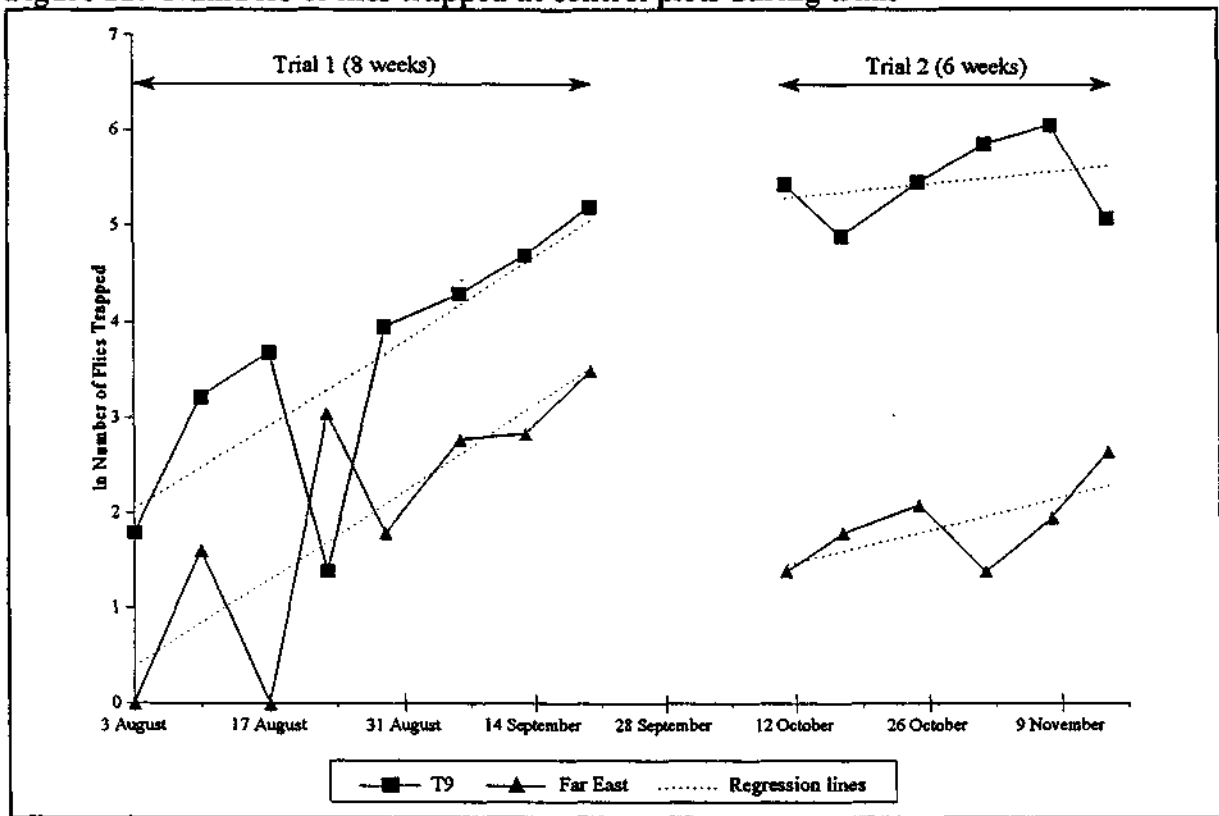
As fruit availability declined, the proportion of fruit infested increased. This is what would be expected as increasing numbers of flies attempt to oviposit in decreasing numbers of hosts. The number of larvae per infested fruit would also be expected to increase as host numbers dwindle. As shown in Figure 8, however, the average number of larvae per infested fruit either decreased or remained steady during the last two weeks of the trial.

Results: Main Trials

Fly Trapping

Log-transformed numbers of *Bactrocera cucumis* trapped at the control plots are shown in Figure 11. Mean trap catches were significantly different at the two sites ($p < 0.001$ for 2-tailed paired sample t-test on transformed values), showing that considerable local variability in fly populations may occur. The transformed values for the two sites increased at the same rate for the first trial (slope for T9 = 0.063, Far East = 0.066, $p < 0.05$ for both), and remained comparatively steady during the second trial (slope for T9 = 0.01, Far East = 0.024, neither significant at the 0.05 level). During the first trial, Far East was sprayed with fungicide while T9 was left unsprayed. In the second trial, both plots were sprayed with fungicide. Fungicide spraying did not appear to cause a decrease in fly populations.

Figure 11: Numbers of flies trapped at control plots during trials



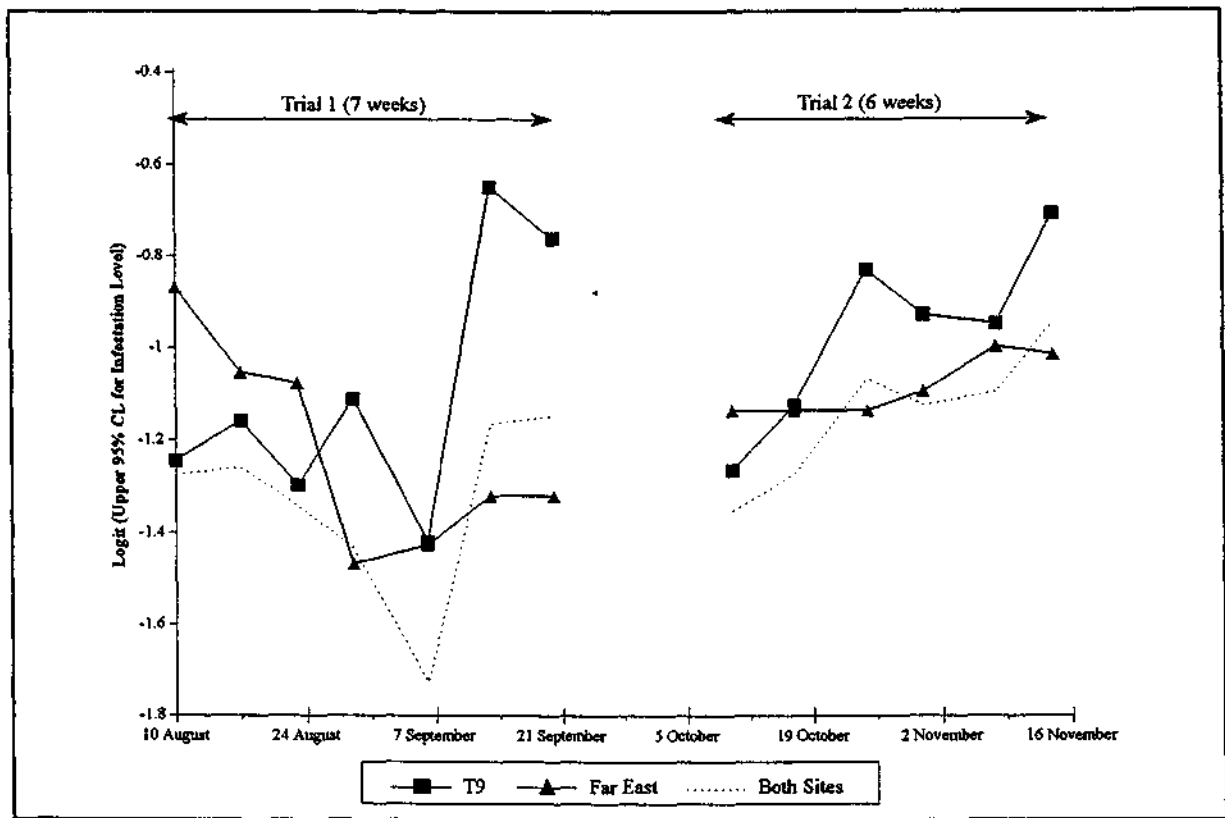
At each plot, numbers of flies caught per trap varied considerably, with traps in shelter surrounding the plots catching far more flies on average than traps within the plots. As in the pilot trial, females were generally more abundant in traps than males.

No fruit flies (*B. cucumis* or other tephritids) were trapped at the export plots during either trial. Shelter in the immediate vicinity of the plots was scarce, but trap locations did include mature sugar cane, a lychee tree in a domestic garden, and an area along a creek approximately 1 km from the export plot (4 traps, trial 2 only). Preharvest control measures (siting plots away from shelter and other hosts, insecticide spraying) appear to have a marked effect on local fly populations.

Infestation of Control Plot Fruit

The proportion of infested export size fruit in the two control plots varied considerably over the trials. Although infestation levels at T9 were generally higher than at Far East (Figure 12), the difference in proportions of infested fruit at the two sites was not quite significant ($p = 0.06$ with a 2-tailed χ^2 test for proportions). Proportions were transformed to logits for linear trend estimation. Because no infested fruit were collected on some weeks during Trial 1, giving an estimated infestation level of 0 for which the logit transformation takes a value of $-\infty$, the upper 95% (one-sided) confidence limits for infestation levels were used. Dashed lines in the figure show logit-transformed infestation levels for both plots combined. At T9, transformed infestation levels increased at approximately the same rate during both trials, although the trend during Trial 1 is not significantly greater than 0. At Far East, infestation levels decreased during Trial 1 and increased during Trial 2. Both trends are significant at the $p = 0.05$ level. As with fly populations, it appears that infestation levels can exhibit considerable local variations.

Figure 12: Infestation levels at control plots during trials

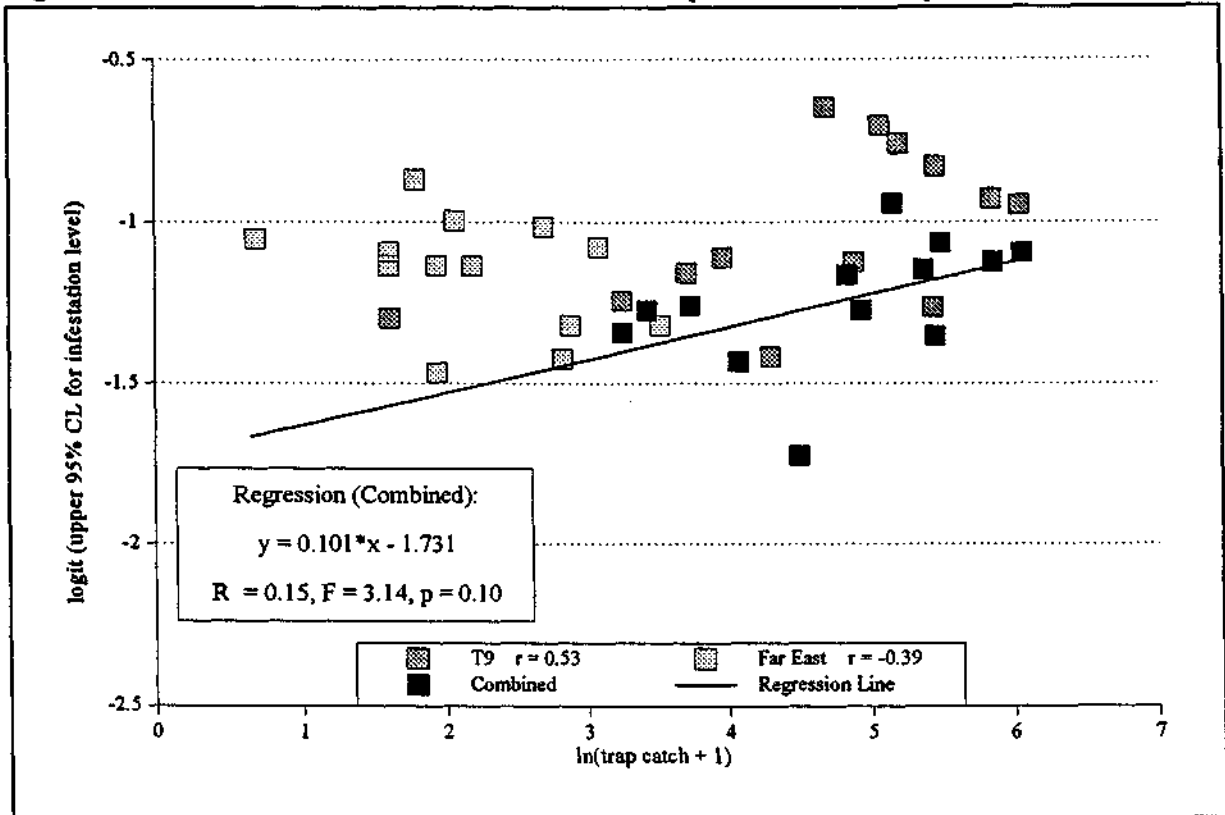


Prediction of Infestation Levels based on Fly Trapping Results

As shown in Figures 11 and 12, considerable local variability may occur in trap catches and measured infestation levels. In part, this may be due to the relatively low attractivity of the orange-ammonia lure for *Bactrocera cucumis*, compared with lures used for other tephritids. Thus, it is not surprising that the control plots do not show a consistent relationship between trap catch and infestation level. During Trial 1, correlations between log-transformed trap catches and

logit-transformed infestation levels (upper 95% confidence limits) were positive for T9 ($r = 0.61$) and negative for Far East ($r = -0.42$). However, during Trial 2, T9 showed no correlation ($r = 0.05$) and Far East showed a positive correlation ($r = 0.55$). Figure 13 shows data for transformed trap catch and infestation levels, together with correlations over both trials. It would appear that trap catches alone can not be used to accurately predict infestation levels, at least within the ranges found at the control plots.

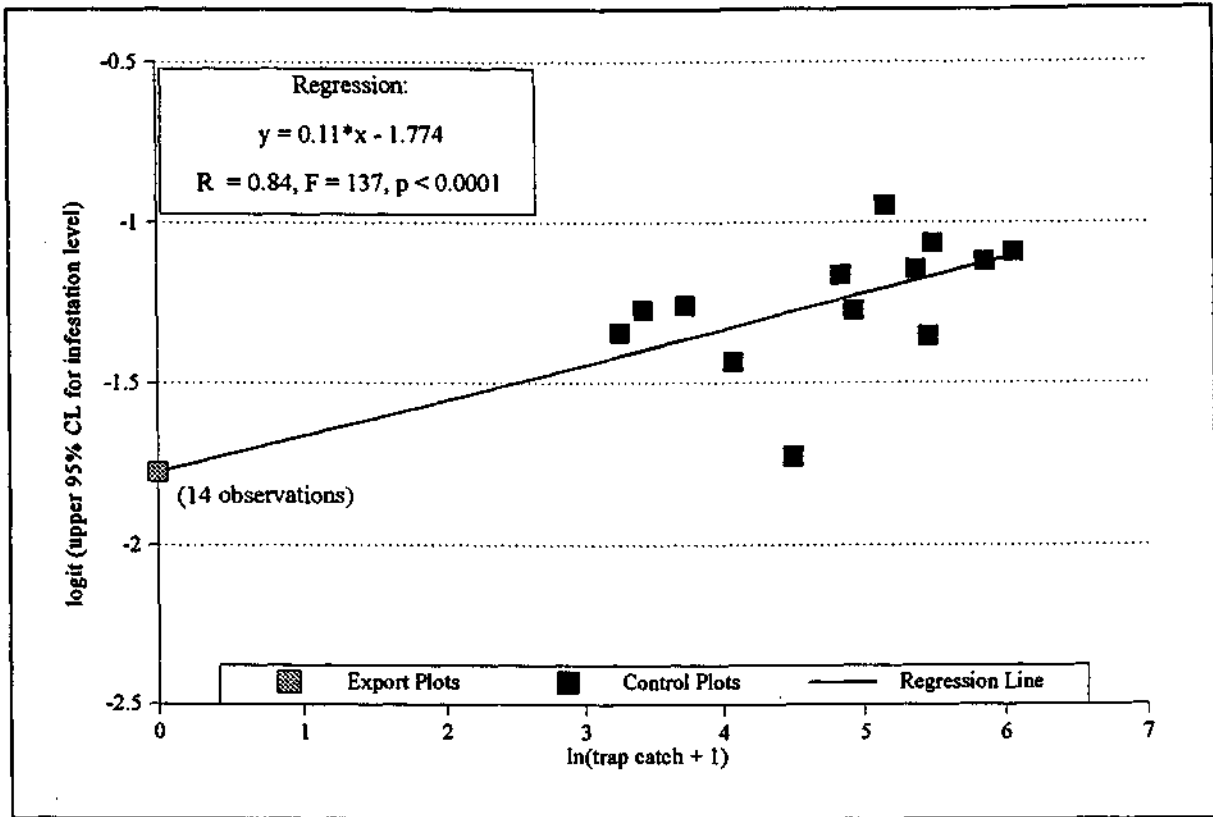
Figure 13: Prediction of infestation level from trap catch: control plots



On a larger scale, however, it may be possible to roughly predict infestation from trap catch. For example, no flies were trapped at the export plots during the trials (14 weeks), suggesting that pre-harvest fly control measures had reduced the local population to an undetectable level. No infested fruit were found in the export plots, essentially giving 14 zero-fly, zero-infestation data points to combine with the control plot data.

Because of the relatively small size and close proximity of the control plots, trap catches and numbers of fruit from the two plots were combined for each date. Fly catches were transformed as $x' = \ln(x+1)$ to avoid a $\ln(0)$ result, and upper 95% (one-sided) confidence limits for infestation level were transformed to logits. Because no infested fruit were found at the export plots, the estimated infestation level is dependent on the sample size used. Samples from the export plots were often 10 times as large as those from the control plots. In order to avoid an artificially high correlation due to sample size effects, infestation levels of the export plots in this example were estimated using a sample size of 180 fruit, equivalent to the maximum combined sample from the control plots. The result of the regression is shown in Figure 14.

Figure 14: Prediction of infestation level from trap catch: large scale differences



The equations of the regression lines are similar, but the significance of the regression is much improved. Thus, it appears that trap catch may be useful for predicting infestation levels on a rough scale. For example, using the regression equation obtained above, a trap catch of 2 would result in a predicted maximum infestation level of 0.022. A single-time trap catch of zero would not give a high level of confidence in near-zero infestation, as fly populations show large local variations and the efficacy of the orange-ammonia lure is low. Other factors such as number and spacing of traps, number of samples, time between trap clearing and lure concentration would all influence the accuracy of prediction.

Larvae In Infested Fruit

Numbers of larvae in infested fruit were extremely variable for both control plots (Figure 15). For T9, $\bar{x} = 48.5$, $s^2 = 1762$, and for Far East, $\bar{x} = 40.4$, $s = 3274$. When numbers were log-transformed, means at the two sites were not significantly different ($p = 0.39$ with 2-tailed t-test). Unlike in the pilot trial, increasing fruit length did not show a clear pattern of increase in larvae per infested fruit.

Overall, transformed numbers of larvae per infested fruit showed a significant increase with date over the two trials ($F = 8.2$, $p = 0.006$), although the predictive ability of the regression is low: $R^2_{\text{adj}} = 0.133$ (Figure 16). Multiple regression using both date and log-transformed fruit length as predictors of larvae per infested fruit was barely significant ($F = 3.592$, $p = 0.04$) and gave little increase in predictive ability ($R^2_{\text{adj}} = 0.139$).

Figure 15: Larvae per infested fruit as a function of fruit length

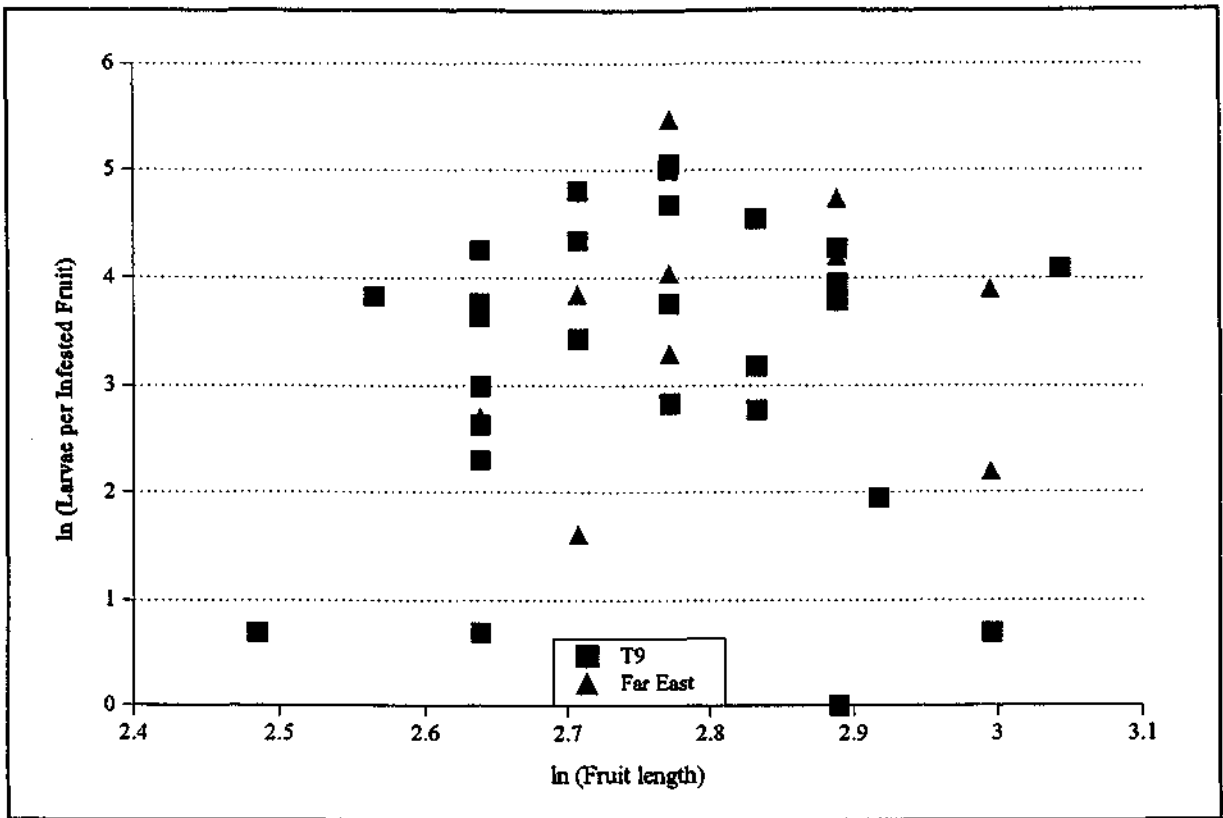
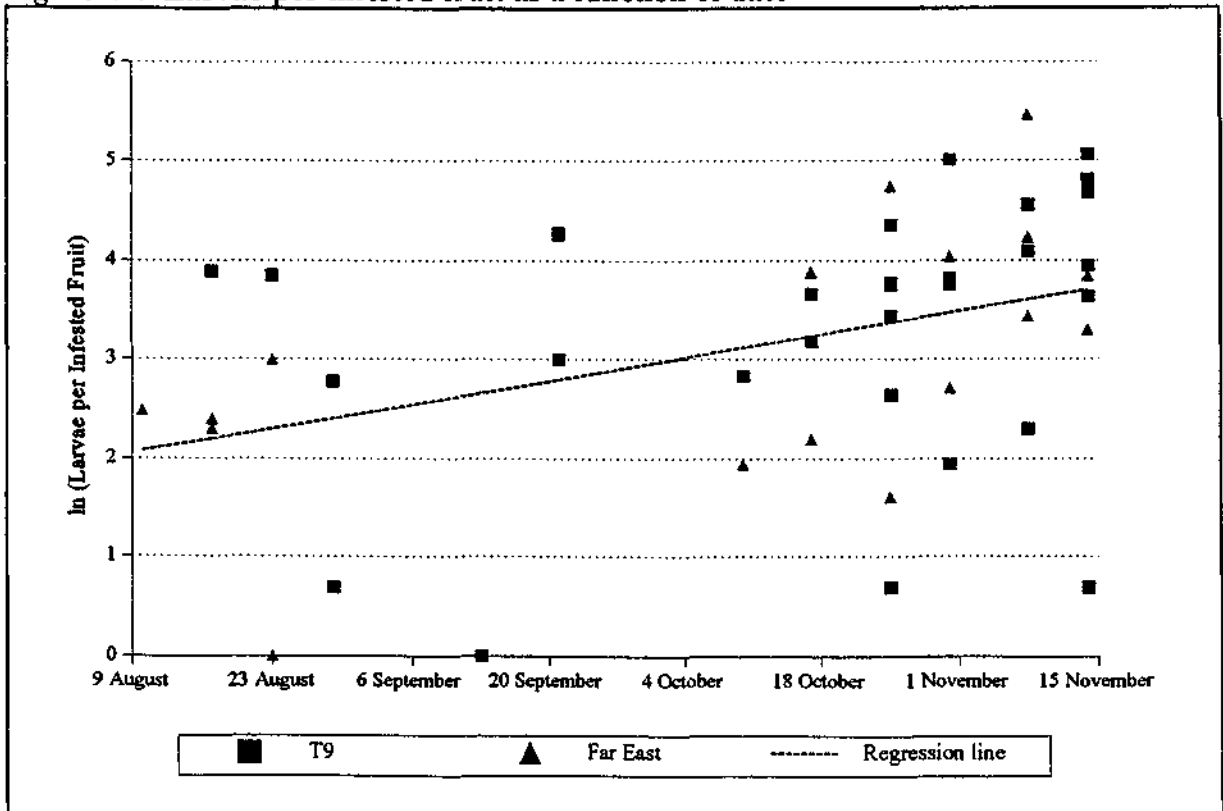


Figure 16: Larvae per infested fruit as a function of date



Infestation Level in Export Fruit and Systems Efficacy

A total of 15,346 export-size fruit were collected from the export plots during Trials 1 and 2 and evaluated for infestation. A further 15,575 fruit from the same plots, prepared for export (washed, sorted, graded, packed and transported to Brisbane) but not dipped in dimethoate solution, were also evaluated for signs of infestation. After holding for a week at 27°C, none of the export-grown fruit showed signs of infestation by *Bactrocera cucumis* or other tephritids.

Assuming the proportion of infested fruit (p) follows a binomial distribution, confidence limits for p can be calculated. The upper 1-sided 95% confidence limits for infestation levels over both trials are shown below. Note that p_{control} is calculated using results from both plots over the whole trial: a total of 1956 fruit were collected, 49 of which were infested.

Table 1: Upper 95% confidence limits for infestation levels across both trials

P_{control}	P_{harvest}	$P_{\text{packing}} = P_{\text{transport}}$
0.0317	1.95×10^{-4}	1.92×10^{-4}

Using the above figures, the efficacy of pre-harvest control measures, P_1 , is 6.2×10^{-3} . Thus, factors such as site selection or modification and insecticide spraying are capable of reducing the infestation level of the export-size crop in the field by 99.4%. This is a lower bound for P_1 , as no infested fruit were found in the export field.

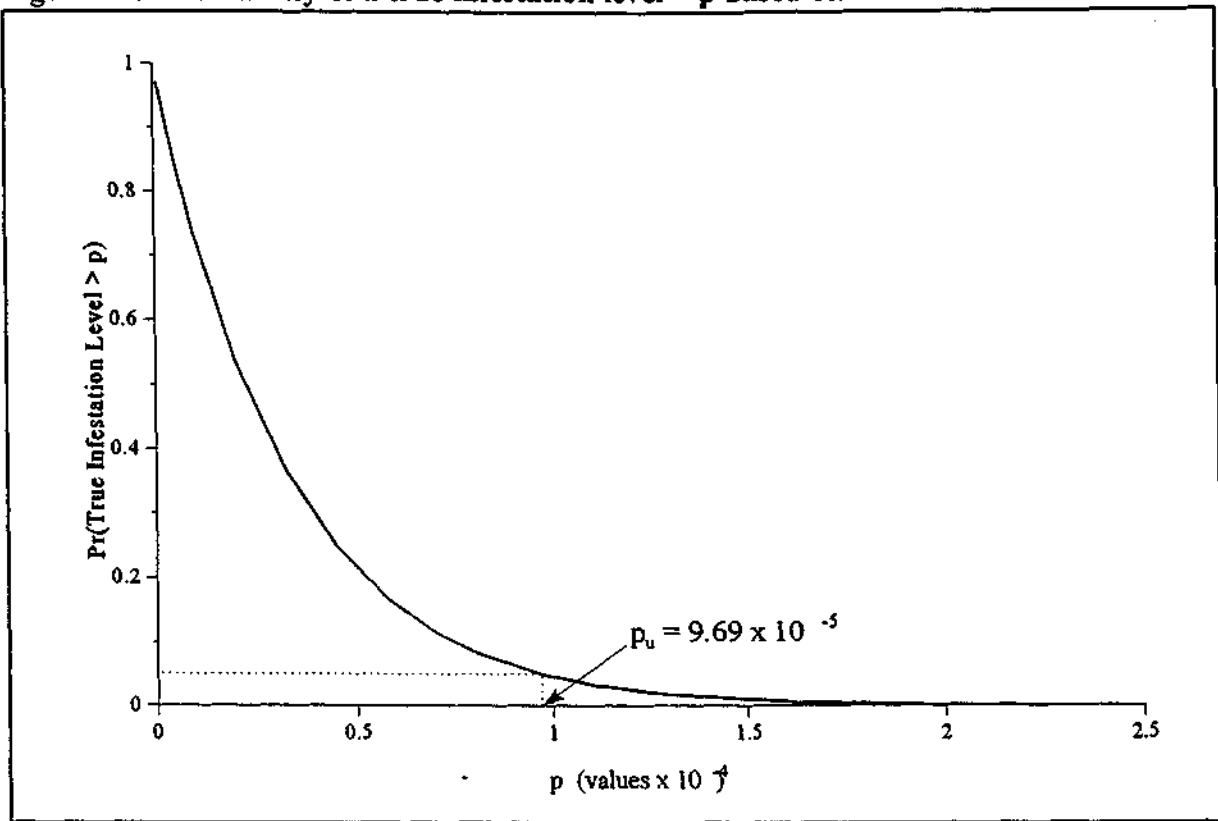
Because no infested fruit were found in either the export field or packhouse samples, the efficacy of the packhouse, P_2 , becomes a ratio of the numbers of fruit examined in each sample rather than a true measure of packhouse efficacy. It is assumed that $P_2 \leq 1$ (e.g. that $p_{\text{packing}} \leq p_{\text{harvest}}$), and that $P_3 = 1$ ($p_{\text{packing}} = p_{\text{transport}}$).

From the results of this study, it appears that pre-harvest control measures are extremely effective in reducing infestation levels of export zucchini.

Because the fruit in the export field and packhouse samples were harvested from the same plots over the course of the two trials, the two sets of samples are assumed to belong to the same "population" of export fruit, and to have been subjected to the same growth conditions, fly populations and infestation pressures. It is also assumed that the infestation level of packed export fruit is not greater than that of ungraded fruit in the field, so that the two sets of samples can be combined to obtain a better estimate of the maximum infestation level in the packed export product over the season.

Again assuming p follows a binomial distribution, an upper 95% confidence limit (p_u) for the proportion of infested fruit can be found such that the probability of the true infestation level being greater than $p_u = 0.05$. With 0 fruit infested out of a total of 30,921 examined, $p_u = 9.69 \times 10^{-5}$ (Figure 17). Based on the results obtained, the likelihood of the true infestation level being $\leq 9.69 \times 10^{-5} = 95\%$.

Figure 17: Probability of a true infestation level $> p$ based on the results obtained



Summary

The major effect of a quality production system is to reduce the infestation level of the exported product. A secondary effect is to also reduce the average number of individuals per infested fruit in the export product. Thus, the values that should be reported are:

- the infestation level of the export product (required)
- the average number of individuals per infested fruit in the export product (optional)

In general, the minimum infestation level of a quality production system will be $p_{transport}$ or the infestation level of the packed product at the export assembly point. As in these trials, $p_{transport}$ may equal $p_{packing}$. Assuming that $p_{control} \geq p_{harvest} \geq p_{packing} \geq p_{transport}$ an infestation level measured at any point along the process is satisfactory. However, as each step in the production system should reduce infestation, it is to the exporter's advantage to measure infestation at the endpoint of the process, rather than earlier.

Data showing the average number of individuals per infested export fruit is not required, but if available, it can help to reduce the strength of total risk management required, particularly if μ in the export product is significantly smaller than maximum published values. In these trials, no infested export fruit were found, so that an estimate of μ in the packed product was not available. As a result, the value of μ in the control plots will be used instead, even though this could greatly overestimate μ in infested packed product.

For risk management purposes, it may not be necessary to measure pre-harvest and packhouse efficacies separately, as done in this paper for example purposes. However, determining the efficacies separately shows the extent to which individual components of the production system contribute to the total efficacy, and may identify components that need improvement or that are unnecessary. For example, in these trials it was shown that pre-harvest fly control measures are capable of reducing field infestation below a level detectable with repeated, high-intensity sampling.

The following section shows how p and μ are used in the generic risk management model (page 6) to calculate the extent to which the production system satisfies the import requirements and determine the strength of any additional risk management.

Risk Management

Using 9.69×10^{-5} as the maximum infestation level of the export zucchini crop over the entire season (see page 18), the need for additional risk management can be determined. Values required for this are:

- the mean and variance (μ and σ^2) of 3rd instar larvae per infested export fruit;
- the probabilities of a fruit arriving at a time and place with suitable climate (C_7) and hosts (C_8), and being disposed of in a high-risk area (C_4); and
- the probability of an individual surviving natural mortality (C_2), transit mortality (C_3), disposal site mortality (C_5), and transmission to a new host (C_6).

Because no infested export fruit were found, the values of μ and σ^2 will be estimated from \bar{x} and s^2 from the control plots. The C values are taken from the PRA for *B. cucumis* (Cowley et al. 1993), with a slight modification of C_2 to exclude egg and early larval mortality. Values used are shown below.

Table 2: Values used to calculate risk management requirements

\bar{x}	s^2	C_2	C_3	C_4	C_5	C_6	C_7	C_8
48.5	1762	0.82	1	0.021	0.8	1	0.21	0.5

The control plot values are taken from T9, which had the highest average number of larvae per infested fruit. The variance is much greater than the mean, suggesting that the number of surviving individuals follows a contagious distribution such as the negative binomial, rather than a random (Poisson) distribution. The average number of individuals surviving, $\bar{x}\phi$, is $48.5 * 0.656$, or 31.8, and the variance is $(s\phi)^2$, or 758.

Assuming equal sex ratios, the probability of a mating pair surviving is $\sum \Pr\{x\} * (1 - (0.5)^{x-1})$, where $\Pr\{x\}$ is the probability of x individuals surviving. Using a negative binomial distribution with $\mu = 31.8$ and $\sigma^2 = 758$ to calculate $\Pr\{x\}$, this probability is approximately 0.95. Note that using Equation 2, page 6, gives a probability of > 0.99 . Thus the use of the Poisson results in a slight error on the side of caution.

When the maximum proportion of infested fruit permissible, M , is calculated as shown in Equation 3, the value is 2.33×10^{-5} . Using the negative binomial distribution, $M = 2.4 \times 10^{-5}$. In either case, this is smaller than the upper 95% confidence limit for packed product infestation level (9.69×10^{-5}), so additional risk management is necessary. This could be accomplished by several different options, as shown in Figure 1. A postharvest treatment will be used as an example.

As the probability of an individual surviving treatment, ϕ , decreases, the probability of a mating pair surviving decreases, and M increases. The required treatment efficacy will be that value of ϕ for which $M = 9.69 \times 10^{-5}$. This value is approximately 0.042. Thus, a suitable postharvest treatment would have to cause at least 95.8% mortality to ensure the MPL is not exceeded.

This assumes that the value of M is known with certainty. This is not the case, however, as considerable variability in survival probabilities is possible. Using a risk analysis program (@RISK) to add variability to the probabilities C_2 - C_8 , confidence values can be calculated for M . Table 3 lists expected values and 95% confidence limits for each probability used in the risk analysis. Probabilities were modelled as standard beta variables, $0 \leq p \leq 1$. The parameters α_1 and α_2 were chosen so that upper and lower 95% confidence limits corresponded to probabilities assumed to be near maximum and minimum values. Where possible, these maximum and minimum values were based on published data. For instance, values for C_7 were determined by calculating the proportion of the country with a suitable climate using a range of temperature tolerances (see Cowley et al. 1993).

Table 3: Probabilities and confidence limits used for risk analysis

	C_2	C_3	C_4	C_5	C_6	C_7	C_8
Expected value	0.82	0.98	0.02	0.81	0.83	0.29	0.29
upper 95%	0.92	1	0.1	0.9	1	0.52	0.5
lower 95%	0.72	0.95	0	0.7	0.5	0.06	0.1

Without treatment, confidence that $M \geq 9.69 \times 10^{-5}$ is approximately 47%. A treatment of $\phi = 0.042$ (see above) increases confidence to $\approx 82\%$, and $\phi = 0.02$ results in $\approx 97\%$ confidence that M is $\geq 9.69 \times 10^{-5}$. Because the overall product of confidence in treatment efficacy and MPL must = 95% (Baker et al. 1990), the confidence in ϕ must be at least 0.95/0.97, or 98%. Thus, a postharvest treatment must have an efficacy $\phi \leq 0.02$ with 98% confidence. Note that this efficacy, equivalent to probit 7.05, is much lower than the traditional probit 9 requirement.

Without knowledge of the infestation level in the export product, the required strength of postharvest treatment would have been that value of ϕ necessary to raise M to 0.005, the infestation level detectable with the 600-unit compliance check. A treatment efficacy of 0.0023 (probit 7.83) gives 97.5% confidence that $M \geq 0.005$. Therefore, confidence in the treatment would also have to be 97.5%.

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APPENDICES

1. RESEARCH PROTOCOL FOR MEASURING INFESTATION LEVELS

2. DATA RECORDING FORMS

SYS 1

SYS 2

SYS 3

SYS 4

SYS 5

RESEARCH PROTOCOL FOR MEASURING INFESTATION LEVELS

Work Plan: Queensland DPI

Control Plots

Location: Bundaberg Research Station
 Cultivar: Zucchini cv "Jet Black"
 Growth Conditions: Plastic mulch (Trial 1 only); trickle irrigation
 Block Specifications: 2 blocks (T9 and Far East), each with 9 rows spaced at 1.5 m intervals x 70 m long.

Treatments:

T9 block	no insecticides	no fungicide (Trial 1 only)	herbicide between rows if necessary
Far East block	no insecticides	fungicides (as used on export plots at Trevor Farmlands)	

Planting date: Week starting 30 May 1994 (Trial 1); 17-18 August 1994 (Trial 2)

Fruit picking: Picking starts approximately 6 weeks after emergence. Research station staff to remove all fruit from all plants every Friday (or other day as negotiated with Dr R.A.I. Drew), preferably at same time each week.

Pre-collection activity

• Divide each block in half; one half of each to be used Trial 1, the other half in Trial 2
• Divide each half-block into 30 plots (see Figure 18)
• Each plot 3 rows x 3.5 m
• Label plots 1 - 60
• Mark plots with coloured tape or flexible poles with flags
• Label each corner plot with its appropriate number
• Label mesh bags with block name and plot number

Fruit collection

• Collect export size fruit (12 - 18 cm) weekly, preferably same day/time each week
• Use measuring stick to select export size fruit
• Select plants by using random number cards (Figures 19a-19b)
• Trial 1: Collect all export-size fruit from randomly selected plant. Collect minimum of 2 export size fruit per plot; if the first selected plant has < 2 export size fruit, then select another plant using the next random number
• Trial 2: Starting at 12:00 on the randomly selected plant and working clockwise, collect the first export-size fruit on the plant. Collect a single fruit from one plant in each row, according the the random number card.
• Collect fruit into labelled mesh bags; one bag per plot
• Collected fruit to be placed in boxes
• Boxes to be insulated in the car

Fruit holding

• Record total number of fruit per plot on Form SYS2
• Place fruit from each plot into a rearing container
• Label each container with block name and plot number
• Fruit to be held at 27 ± 1 °C for 6 days after picking
• After 6 days, each fruit to be examined individually
• Record number of larvae in each infested fruit on Form SYS3
• Record number of infested fruit per plot on Form SYS2

Figure 18 shows the layout of the control plot sites, including trap locations, for Trial 1. In Trial 2, the other halves of the plots were used.

Figure 18: Layout of control plots at Bundaberg Research Station

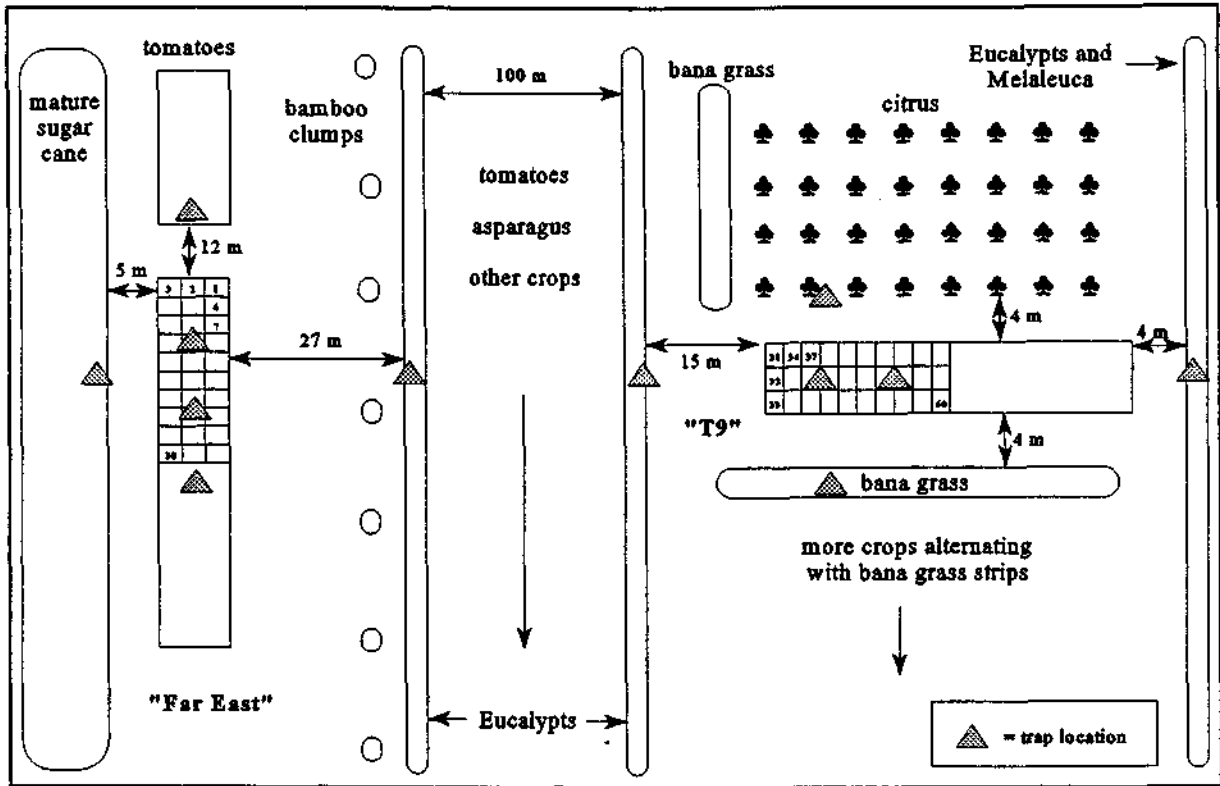


Figure 19: Random number cards used for control plot sampling

19a: Trial 1

Far East: Week 1*		
	Row	Plant
Plant 1	1	2
Plant 2	2	8
Plant 3	3	4
Plant 4	1	7
Plant 5	3	9
Plant 6	1	3
Plant 7	2	1
Plant 8	3	6
Plant 9	3	5

19b: Trial 2

Far East: Week 1		
Row 1	Row 2	Row 3
2	6	9
8	1	5
6	7	2
7	5	6
3	4	4
9	2	3
1	8	8
5	3	7
4	9	1

* different cards were used each week

Export Plots - Trevor Farmlands

Seed planted 27 May 1994 (Trial 1) and 17-18 August 1994 (Trial 2).

Start collection approximately six weeks from emergence.

Pre-collection activity

- Mark 3 bands of export block, each 25 m long; one at each end and one in the middle of the block (Figures 20-21)
- Trial 1: Export block has 54 rows. Divide each band into 45 plots, each plot 6 rows x 5 m, label plots 1-135 (Figure 20)
- Trial 2: Export block has 50 rows. Divide each band into 50 plots, each plot 5 rows x 5 m, label plots 1-150 (Figure 21)
- Mark plots with coloured tape or flexible poles with flags
- Label each corner with plot number
- Label mesh bags with block name and plot number

Fruit collection

- Collect export size fruit (12 - 18 cm) weekly, preferably the day before commercial picking and same day/time each week
- Use measuring stick to select export size fruit
- Select plants by using random number cards (Figure 22)
- Trial 1: Collect from the randomly selected plant and its nearest 4 neighbours. Sample nearest neighbours in a clockwise direction. Continue fruit collection until a minimum of 9 fruit per plot is reached. All export size fruit must be taken from a plant once sampling has started.
- Trial 2: Starting at 12.00 position and working clockwise, collect the first export size fruit from each plant selected. Collect 2 export size fruit from 2 randomly-selected plants in each row of the plot (approximately 10 fruit per plot).
- Collect fruit into labelled mesh bags; one bag per plot.
- Put bags into boxes
- Boxes to be insulated in car

Fruit holding

- As for control plots, except that Form SYS4 used instead of SYS2

Figure 20: Layout of export block at Trevor Farmlands, Trial 1

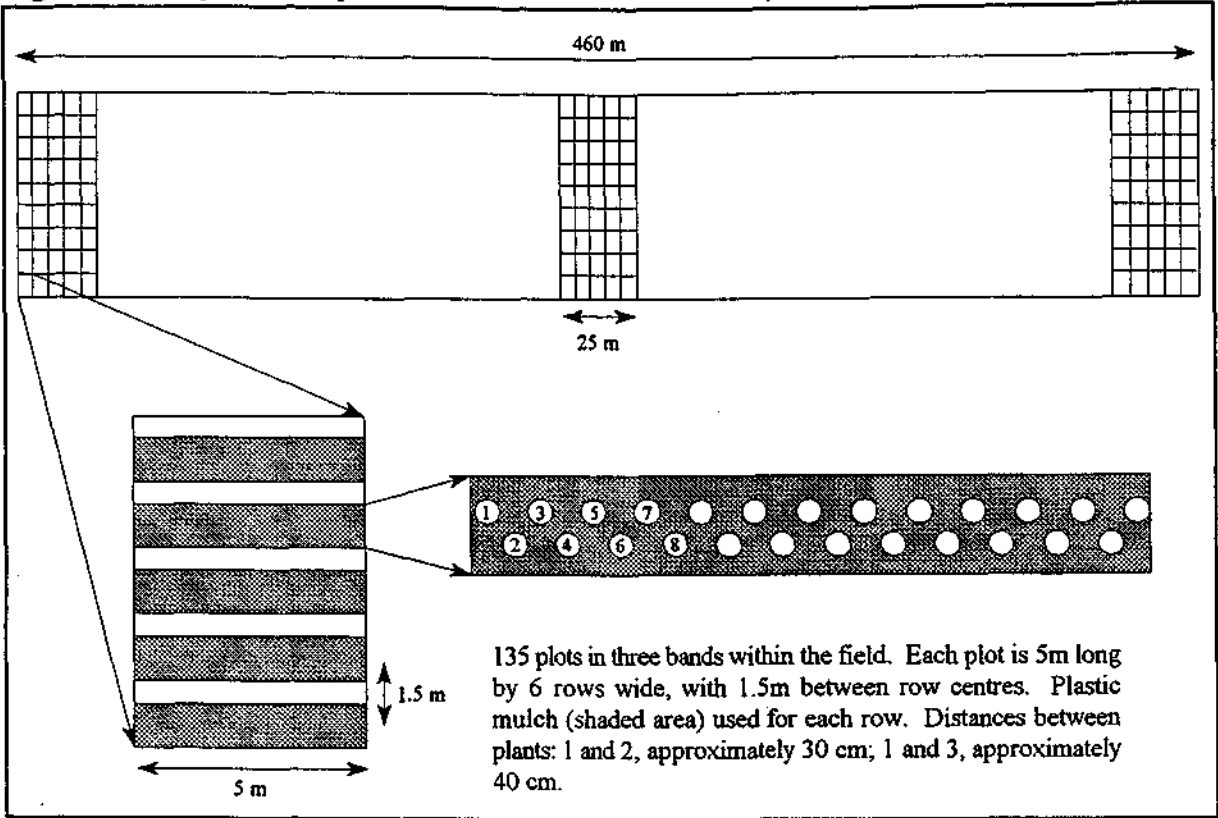


Figure 21: Layout of export block at Trevor Farmlands, Trial 2

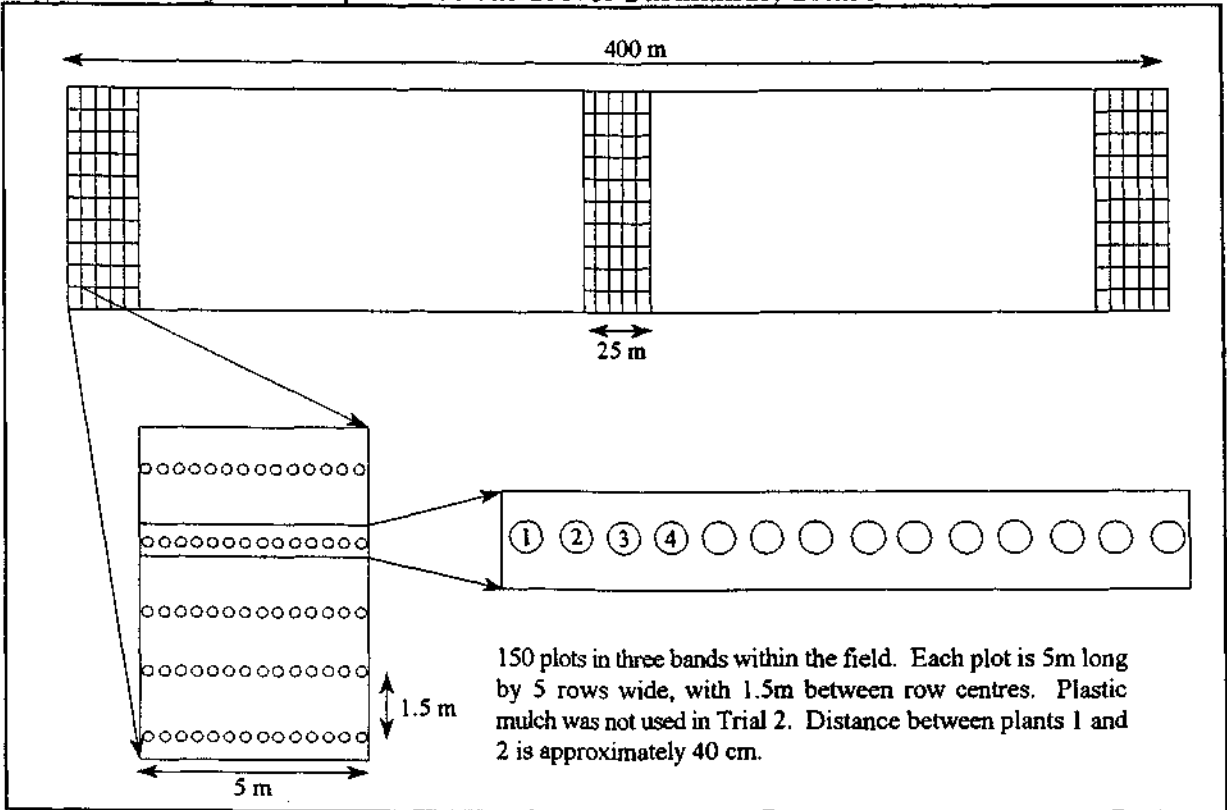


Figure 22: Random number cards for export trials

22a: Trial 1

Trevor Farmlands: Week 1		
	Row	Plant
Plant 1	1	6
Plant 2	4	25
Plant 3	1	16
Plant 4	5	11
Plant 5	3	2
Plant 6	3	8
Plant 7	4	9
Plant 8	1	1
Plant 9	2	24
Plant 10	1	15
Plant 11	3	10
Plant 12	5	20
Plant 13	4	19
Plant 14	3	18
Plant 15	5	9
Plant 16	4	6
Plant 17	1	10
Plant 18	5	8
Plant 19	2	13
Plant 20	5	21

22b: Trial 2

Trevor Farmlands: Week 1				
Row 1	Row 2	Row 3	Row 4	Row 5
7	5	12	2	4
14	1	11	9	2
12	13	4	11	6
8	4	13	1	7
3	7	14	7	10
2	3	7	13	3
6	10	3	4	5
4	11	9	14	11
10	12	5	12	9
1	9	2	3	8
5	2	6	6	14
11	8	10	5	12
13	6	8	10	1
9	14	1	8	13

Packed Product Samples - Trevor Farmlands

Fruit for these samples was grown in the same export plots as the export field samples (Figures 20 & 21), picked, graded and packed by staff at Trevor Farmlands as part of the normal harvesting and packhouse routine. These fruit were generally picked the same day or day after the export field samples were picked. Due to high numbers of fruit collected (approximately 4,000 per sample), only three samples of packed product were planned for each trial. Due to Trial 2 ending earlier than expected, only 2 packhouse samples were actually assessed.

Trial 1: one shipment in 2nd, 5th, and 8th weeks.

Trial 2: one shipment in 2nd, 4th, and 6th weeks.

Fruit selection

<ul style="list-style-type: none">● 40 cartons of export quality and export size fruit, packed into meshed export cartons, but not passed through dimethoate dip.
<ul style="list-style-type: none">● Cartons clearly marked with block number and letters DPI (coloured labels to be provided by DPI for this)
<ul style="list-style-type: none">● The 40 cartons are to be held together as a unit with binding tape or similar (not shrink-wrapped unless this is used with export fruit)
<ul style="list-style-type: none">● Cartons to be transported to Brisbane by Lindsay Bros Transport, under same conditions as export fruit.

Fruit holding

<ul style="list-style-type: none">● Fruit to be held in rearing containers.
<ul style="list-style-type: none">● Containers to be labelled
<ul style="list-style-type: none">● Fruit to be held at 27 ± 1 °C for 7 days after picking
<ul style="list-style-type: none">● After 7 days, each fruit to be examined individually
<ul style="list-style-type: none">● Record number of larvae in each infested fruit on Form SYSS
<ul style="list-style-type: none">● Record total number of fruit on Form SYSS
<ul style="list-style-type: none">● Record total number of infested fruit on Form SYSS

Trap Monitoring: Bundaberg Research Station and Trevor Farmlands

- Control
 - T9: 4 traps in shelter surrounding crop, 2 traps inside crop
 - Far East: 4 traps in shelter surrounding crop, 2 traps inside crop
- Trevor Farmlands
 - Trial 1: 6 traps in shelter adjacent to crop, one trap on crop perimeter, one trap in a Lychee tree in a domestic garden 50m from crop
 - Trial 2: 8 traps around perimeter of crop, 4 traps along a creek ~1 km from the crop

Procedure

● Record location on map
● Label traps with block name and trap number
● Collect all <i>Bactrocera</i> spp. into labelled tubes of alcohol weekly
● In lab, identify, count, and sex all <i>Bactrocera</i> ; record all <i>B. cucumis</i> on Form SYS1.

Traps to be baited one week before first collection, and rebaited after each collection. Trap collection started 10 August for Trial 1 and 11 October for Trial 2.

In order to maximise the likelihood of catching flies, traps were hung in shelter where available. At the research station, shelter was provided by sugar cane, bana grass (similar to sugar cane in structure), eucalypt trees and citrus plantings. At the export block used in Trial 1, shelter was provided by mature sugar cane (cut mid-trial), a grass verge, scattered eucalypt trees, and a domestic garden approximately 50m from the crop. At the export plot used in Trial 2, there was no shelter immediately adjacent to the plot, so in addition to the 8 traps used in Trial 1, four additional traps were set out in a creekbed approximately 1 km from the plot.

SYS 1

Numbers of *Bactrocera cucumis* in bait traps

Site: _____

Week 1			Week 2		Week 3		Week 4		Week 5		Week 6		Week 7		Week 8	
Date:	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Trap No.																

SYS 2

Control Block Summary: Infested Fruit per Plot

Date:

"T9"			"Far East"		
Plot No.	No. Fruit	No. Inf. Fruit	Plot No.	No. Fruit	No. Inf. Fruit
1			31		
2			32		
3			33		
4			34		
5			35		
6			36		
7			37		
8			38		
9			39		
10			40		
11			41		
12			42		
13			43		
14			44		
15			45		
16			46		
17			47		
18			48		
19			49		
20			50		
21			51		
22			52		
23			53		
24			54		
25			55		
26			56		
27			57		
28			58		
29			59		
30			60		

Trevor Farmlands Summary: Infested Fruit per Plot

Date:

Plot No.	No. Fruit	No. Inf. Fruit	Plot No.	No. Fruit	No. Inf. Fruit
1			31		
2			32		
3			33		
4			34		
5			35		
6			36		
7			37		
8			38		
9			39		
10			40		
11			41		
12			42		
13			43		
14			44		
15			45		
16			46		
17			47		
18			48		
19			49		
20			50		
21			51		
22			52		
23			53		
24			54		
25			55		
26			56		
27			57		
28			58		
29			59		
30			60		

Trevor Farmlands Summary: Infested Fruit per Plot

Date: _____

Plot No.	No. Fruit	No. Inf. Fruit	Plot No.	No. Fruit	No. Inf. Fruit
61			91		
62			92		
63			93		
64			94		
65			95		
66			96		
67			97		
68			98		
69			99		
70			100		
71			101		
72			102		
73			103		
74			104		
75			105		
76			106		
77			107		
78			108		
79			109		
80			110		
81			111		
82			112		
83			113		
84			114		
85			115		
86			116		
87			117		
88			118		
89			119		
90			120		

Trevor Farmlands Summary: Infested Fruit per Plot

Date: _____

Plot No.	No. Fruit	No. Inf. Fruit	Plot No.	No. Fruit	No. Inf. Fruit
121					
122					
123					
124					
125					
126					
127					
128					
129					
130					
131					
132					
133					
134					
135					
136 *					
137					
138					
139					
140					
141					
142					
143					
144					
145					
146					
147					
148					
149					
150					

* Numbers 136 - 150 used for Trial 2 only

