Research reports

Pilot study at Cowra of intra-town dynamics of Queensland Fruit Fly (*Bactrocera tryoni* (Froggatt)) populations based on trap catch data

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Abstract
We revisit data previously reported by James (1992) relating to variation in Queensland fruit fly numbers at 30 trap locations on a 400 m grid in the town of Cowra during summer of 1991/2. Wild fly numbers were linked to aspect/slope interaction and aspect/altitude interaction. CLIMEX indices and anemometer readings were not significantly linked to wild fly numbers. Sites with a northern aspect, higher slope, lower altitude and preferred host trees appear to be favourable sites for wild flies, particularly with shorter day-length. Wild fly populations were not influenced by sterile numbers, confirming the previous perception. Sterile fly numbers were not linked to any of the examined factors.

Introduction

Queensland fruit fly (Qfly) *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) has long been known as a serious pest of the Australian fruit industry causing millions of dollars damage annually (Anon. 1997). Currently fruit flies are a concern to Australia's $6.9 billion horticultural industries (Hyam 2007). In a national stocktake, Oliver (2007) reported it was anticipated that $128.7 million would be spent on fruit fly related activities in Australia from July 2003 to June 2008. Qfly eggs are laid into fruit near the surface and the larvae burrow to the centre of fruit causing it to spoil. Qfly is a native of Australia and has been introduced to several Pacific islands. Other trading partners want to remain free of this pest and strict quarantine regulations apply to fruit exported from Australia (Anon. 1997).

Qfly occurs widely throughout eastern Australia in up to 243 known hosts (Hancock *et al.* 2000) but with diminishing intensity in cooler and southern areas. In these marginal areas, outbreaks occur throughout spring and summer tapering off through autumn in response to changes in temperature and humidity (Yonow and Sutherst 1998, Yonow *et al.* 2004).

Controlling Qfly is an expensive process. The NSW Department of Primary Industries spends nearly $2 million annually to control this pest (Walker personal communication). These control programs could be significantly better targeted if over-wintering sites were characterized and identified. Over-wintering sites are typically those areas where adult flies survive during winter, where populations first break out at the start of the season and where populations linger at the end of the season. Control techniques such as the sterile insect technique (SIT), cover sprays with systemic pesticides and bait sprays ('lure and kill') might be used to saturate those over-wintering areas in autumn to reduce the number of surviving adults and in spring to retard population re-establishment.

Despite considerable research in the laboratory on how environmental parameters affect Qfly, there have been very few field studies in this area over recent years. The parameterization of Qfly population movement, within towns, through the season has the potential to reduce the need for widespread control measures by targeting spots where Qfly will most likely occur and survive.

With the advent of better statistical software, it was possible to revisit historical data and re-examine the influence of environmental factors such as host plant, altitude and aspect on Qflies. In this paper, we re-examine insect counts from a trial previously reported by James (1992) with the aim of verifying his original results and to discover any new environmental information from the original work. The intention of this paper was also to identify areas preferred by Qfly.

Methods

The release of sterile Qfly and the monitoring of wild and sterile Qfly in Cowra, New South Wales, was reported by James (1992). In general, pupae were produced at Gosford and covered in fluorescent dye for identification after release. Pupae were irradiated in normal air with 70–74 Gy at the Australian Nuclear Science and Technology Organisation facility in Lucas Heights, a suburb of Sydney. After irradiation, consignments were transported by road courier to Cowra, a distance of 350 km. In total, 37.08 million pupae were received at Cowra, and after emergence rates were considered, it was estimated that 8.34 million males were released with 2330 sterile males recaptured. Sterile flies were released weekly from 10 October until 5 December, 1991 (nine releases), and from 6 February until 7 May 1992 (13 releases) from 45 L plastic rubbish bins. There were 15 release sites on an 800 m grid across Cowra. In the weekly cycle of the program, pupae were usually received on day one and placed in the bins, emergence began on day three, adults were held for two days and released on day five or six. The bins were cleaned and dried on day six or seven every week, with the next batch of pupae being received on the following day. During the emergence from its pupal case, the adult expands its ptilinum to burst the pupal case to facilitate escape, and during this process the moist ptilinum contacts the dry dye crumbs on the pupal case. The ptilinum, with dye crumbs, is withdrawn back into the head after emergence of the adult, permanently marking the fly. The marker dye is known not to degrade for at least six months (Dominiak unpublished data). Emerged flies were provided water and white sugar cubes.

Cowra had a network of 30 Lynfield traps (Malathion and cuelure fruit fly traps) (Cowley *et al.* 1990) set out on roughly a 400 m array to monitor the sterile and wild male Qfly populations – there is no reliable female trap (Dominiak *et al.* 2003a, 2006). Trap sites were well separated from sterile fly release sites. Monitoring was conducted from 15 October 1991 to 2 September 1992 (39 trapping events in 48 weeks). Trapped flies were collected primarily at weekly intervals and were examined using a binocular microscope fitted with an ultraviolet filter in the light source. Sterile flies were identified by distinct traces of fluorescent dye observed adhering to the body or ptilinum. Flies without obvious dye were classified as wild flies (James 1992, Dominiak *et al.* 2000). Insufficient

resources were available to perform dissection of testes or ptilinum. In some species (Koyama *et al.* 2004), data exists which indicate that testes of irradiated flies were smaller than wild flies to backup the ptilinum dye examination, however this data was not available for Qfly in 1992. Wild and sterile trap catches were entered into a Microsoft Access database.

For analysis, trap monitoring and release sites were characterized by geographic location, altitude, slope, aspect and host tree. Location coordinates (*x, y*) were expressed as Australian Metric Grid References (AMG), zone 55, using the Australian Geodetic Datum 1966. Coordinates were captured through on screen digitizing over the New South Wales Department of Lands (LPINSW) Digital Cadastral Database (DCDB) as a backdrop in the GIS package (ESRI ArcView v3.2a). The resultant georeferenced dataset (called a shapefile) was used to determine the altitude, slope and aspect of individual trap and release sites. The trap and release site shapefile was intersected with a one second (25 m grid) Digital Elevation Model (DEM) to determine the altitude of each site. Slope and aspect surfaces were calculated from the one second DEM using ArcView Spatial Analyst which fits a plane to the z values of a 3 × 3 kernel around the processing (centre) cell. The direction the plane faces is the aspect for the processing cell. The slope for the cell is calculated from the 3 × 3 neighbourhood using the average maximum technique (Burrough and McDonnell 1998).

Preferred host trees (trees where traps were hung) such as stone fruit trees were not available in all trap sites. Fourteen host species were used at the 30 monitoring sites but it was expected that traps in some host trees (such as peaches) would collect more flies than in trees such as bottlebrushes, an Australian native bush with no fruit. There were insufficient trap sites to analyse the fourteen host tree species as such and so they were scored as 1 (unattractive e.g. palm tree), 2 (mildly attractive non fruiting e.g. *Prunus*) and 3 (attractive, e.g. stone fruit). This score was fitted as a covariate.

Climatic data were obtained from SILO (Jeffrey *et al.* 2001), and wind effect from the Department of Natural Resources (DNR) Cowra Research Centre. Daily data was entered into the CLIMEX model (Yonow and Sutherst 1998) and the model run, providing the various indices of moisture, temperature and light. These indices were however dominated by the change in day length over the period and were excluded from the model.

Analysis

Three analyses were performed using a log scale: wild fly counts, sterile fly counts and a joint analysis. The model included the explanatory location variables (altitude, slope, aspect and host), time variables (day length, cubic spline of time and some weather variables) and interactions. These were tested in a mixed model with trap-site and time being fitted as random terms. Terms were tested in an analysis of variance using ASReml (Gilmour *et al.* 2002) and non-significant terms were omitted to produce the models reported here. Weather variables were not available for all times and were tested on reduced data sets for which they were available.

Results

Wild flies

Model based on log(count + 1) After omitting times when climatic data was not available and missing counts, there were 869 counts of wild flies representing the 30 traps and 29 occasions available for analysis (most trap inspections occurred on a weekly basis). A preliminary analysis fitting traps and dates as fixed factors shows there are significant differences in both, with changes due to time being greatest. The retransformed simple means over traps are displayed in Figure 1. Further models were then fitted to investigate whether the trap and time differences were associated with any of the explanatory variables.

The factors that significantly related to the counts of wild flies are listed in Table 1. They form three groups: those that related to time (day length and cubic spline of time), those that relate to location (altitude, aspect, slope and host) and interactions. The CLIMEX indices and the anemometer reading did not contribute to the model and these factors were excluded from further analyses which were then based on 39 occasions. The North-South component of aspect was significant but not the East-West component. Host trees at trap sites were not quite significant ($P > 0.06$).

The cubic spline for time together with day length defines the overall pattern of increasing counts early in the season and decreasing counts later. However, day length was the dominant term being involved in an interaction with slope. The model predicted that locations with a northerly aspect, higher slope, lower altitude with fruit trees were the most favourable sites.

An initial analysis indicated that there was no significant trend associated with the *x,y* coordinates. There was a positive correlation between traps which declined as distance between traps increased but the correlation was not significant, indicating that the traps were far enough apart to be regarded as independent.

We note that wild flies were present when sterile flies were released, but that the numbers remained relatively low over the first nine weeks when sterile flies were being released. There was a gap in trap monitoring and release of sterile flies over the Christmas period. When measurement resumed in January (week 12), wild fly

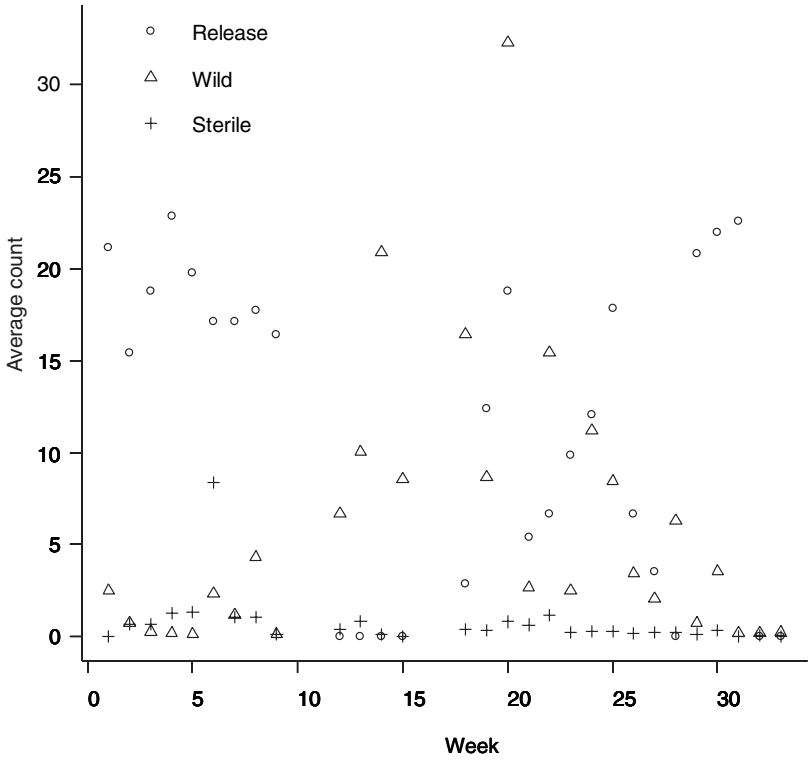

Figure 1. Changes in average counts of sterile (+) and wild (△) Qfly during the 23 October 1991/2 at Cowra. The relative numbers of sterile flies released during the preceding period is also indicated (○).

Table 1. Analysis of Variance for the log(count + 1) for wild flies.

Factor	Regression Coefficient	Significance
Constant	1.664 ± 5.755	NA
Location		
Altitude (z)	−0.015 ± 0.004	NA
Slope	0.771 ± 0.136	NA
Aspect	1.671 ± 0.671	NA
Host Tree Type	0.174 ± 0.088	NA
Aspect*slope	0.315 ± 0.115	0.008
Aspect*Altitude (z)	−0.019 ± 0.007	0.008
Time		
Day length	0.055 ± 0.641	NA
Time(months)	−0.061 ± 0.372	0.003
Location.Time		
Day-length*Slope	−0.039 ± 0.009	<0.001

NA indicates the test is not presented because the term is involved in an interaction.

counts had increased and continued to do so. Sterile fly releases also resumed in February. It is impossible to identify whether the sterile fly releases early in the season contributed to the low wild fly counts in this period.

Sterile flies

Model based on log(count + 1) A similar modelling process was performed on the numbers of sterile flies trapped. There was significant variation between traps and between times with time having greater effects than trap. However, sterile fly counts were low compared to wild fly counts. There was an overall decline with time and other explanatory variables were not significant. In particular, the sterile fly count was not significantly influenced by sterile fly releases. The between-trap variability was greater in summer and autumn than in spring. Since none of the climatic variables were significant, this model was confirmed on the total data (including those times when climatic data was unavailable).

Model for the wild/sterile fly relationship The counts varied independently. The number of sterile flies captured was not a predictor of wild flies captured.

Discussion

Concerning sterile flies, the lack of relationship between sterile fly releases and sterile fly counts may be explained by the viability problems of the sterile flies after road transport to Cowra. These problems led to changes in the transport protocol for subsequent releases to minimize transport losses (Dominiak *et al.* 2000, 2003b). Despite these changes, Dominiak *et al.* (2007) reported a 16.7% decline in emergence using largely air transport. The trap sites were not near the release sites, so the trapped counts indicate that the sterile flies had dispersed but were only present at a low level.

Concerning wild flies, the general pattern of wild Qfly numbers was as expected with low numbers early, high numbers over summer as fruit ripens and decreasing numbers in autumn (Figure 1). These numbers were unaffected by the sterile fly program. The sterile fly program may have been ineffective because releases began after the wild flies had broken out of their over-wintering mode and because releases were suspended in early summer over the Christmas period during which time wild fly numbers rose without any restriction. The suspension of releases coincided with start of the second generation and the associated wild matings. The start to the release program (October) is now generally considered to be too late.

The dominant source of differences in wild fly counts were related to the strong seasonal pattern which was largely independent of trap location effects. The locality covariates of importance were host tree type, aspect, slope and altitude with higher counts associated with traps in fruit trees, a northerly aspect, steeper slope and lower elevation. These covariates did not explain all the spatial variation. Location of traps indicated by latitude and longitude coordinates had no effect on numbers.

In all wild fly analyses, the aspect*altitude interaction was significant. This indicates that these interactions are important contributors to wild fly populations probably because of their impact on the micro-climate. The climatic data we used was at a macro level and is unlikely to assist with micro-climatic analysis. Traditional understanding of fruit fly populations has been based on temperature and humidity; the possible role of altitude (range from 286 to 359 m above sea level) and aspect in locally modifying temperature and humidity has not been considered before and this is worth further examination in the future.

With only 29 time points over 33 weeks of the release program, it is not possible to tease out the separate influences of temperature and moisture, both as rainfall and as humidity, on wild fly populations as they are all seasonal variables. When examined by themselves in this study, the environmental factors of day-length, rain and barometric pressure were significantly associated with wild flies counts. Rainfall is usually associated with low-pressure systems so it could be inferred that rain was dependent on barometric pressure although this was not tested.

Regarding the interactions, Qfly counts increased with slope, but less so with long days (slope 0.39 (0.19)) when day-length was 10 (15) hours. This slope increased to 0.31 on North (South) facing sites. This aspect effect is tied up with altitude such that higher sites had lower counts if north facing than aspect *per se* would indicate. We infer that altitude and the aspect probably affected the micro-climate resulting in higher counts at some sites at the less favourable times.

One area that the authors hoped to examine was the possible issue of wind or wind assisted movement. Anemometers or wind was not a significant factor in this model. The average weekly wind run for Cowra for the examination period was 644 km, equating to 3.8 km hr^{-1}. The highest wind run in any week was 1036 km with the highest daily wind run of 319 km, equating to 13.3 km hr^{-1}. Dominiak *et al.* (2003b) suggested wind did not move fruit fly populations, however the issue of wind assisted movement of flies remains unclear. Given the limitations of this review, our results did not support the theory that fruit flies were moved by wind.

CLIMEX has been a useful program to provide weekly averaging and the provision of many indices (Dominiak *et al.* 2000, 2003a,b). It was hoped that this system would be useful in providing thresholds or indices to indicate wild fly activity. However, the available data was not on a site but a whole town basis and proved inadequate.

Interpretation of individual regression coefficients is difficult because of the high level of association between them. However, we conclude that wild fly numbers were affected by the seasonal variables and by location variables of altitude, slope, aspect and host tree type.

There were significant week to week changes which were not explained by the model. There were also significant correlated trap effects, which were not explained by host tree type, altitude, slope and aspect. These covariables point to northerly aspect, high slope, lower altitude fruit trees as being the most favourable location. As day-length increased, the impact of slope reduced.

Wild/sterile fly relationship

The analysis indicated that sterile flies had no significant impact on the number of wild flies. However the smaller between-trap variability for sterile flies in spring, along with the low numbers of wild flies in these first nine weeks, might suggest that the sterile flies suppressed wild fly population early in the season. This current analysis was not available when James (1992) was written, but our current analysis supports the conclusions of James. If a sterile release program is to succeed, the releases must start early to minimize the number of wild offspring as a result of the matings following the over-wintering flight. James acknowledged that the Cowra sterile releases started after the over-wintering flight and this lateness penalized the control program. Dominiak *et al.* (2003c) demonstrated that sterile release before the over wintering flight could greatly reduce the wild fly population during the following season.

The program was further penalized by the low quality of sterile flies as a result of road transport of irradiated pupae. Our analysis indicates that fewer sterile flies were caught after late spring, presumably as a result of the combination of summer heat and vibration for five hours on road transport prior to release. This decline in sterile flies in early summer, which is the start of the peak wild fruit fly season, further doomed the control program.

We could find no contributing factors to problems with sterile fly populations in our analysis. Therefore sterile numbers captured were likely to be due to the many problems reported by James (1992) with some consignments having a high death rate on delivery. Dominiak *et al.* (2007) also identified transport stress as a major contributor to decreased emergence at release sites. This decline in viability is likely to be amplified by rearing and field delivery stresses, further diminishing sterile fly survival and recapture. Notwithstanding these problems, there were many outcomes from this Cowra work which have been utilized to improve subsequent sterile release programs (Dominiak *et al.* 2000).

In summary, the aim of this pilot study was to assess if historic data could be re-evaluated to find new information to increase the understanding of Qfly, and in this we were successful. There were many factors and many relationships between factors which made it difficult to find clear contributors to an understanding of the wild fly population dynamics. This study did not cover an entire year and the results may be biased because the early season activity was not recorded. Our results should be regarded as tentative only. This re-evaluation supports historical concepts (Dominiak *et al*. 2006, Yonow *et al.* 2004) of temperature and moisture, as evaporation and rainfall, as being important determinants of wild fly population. We conclude that the original assessment by James was correct and that the sterile flies did not significantly decrease the wild populations. The original comments about the variable quality were supported by our findings that recaptured sterile flies were not related to number of pupae received at Cowra for adult release. The impact on wild fly numbers of altitude, with its interaction with day-length, slope and aspect, was a new finding that has not been previously reported. Sites with a northern aspect, high slope, lower altitude with host trees appear to be favourable sites. The issue of the impact of altitude and aspect on Qfly populations should be examined in other locations to check if these factors play a role in other fruit fly habitats.

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