551

P-001

BUREAU OF SUGAR EXPERIMENT STATION
QUEENSLAND, AUSTRALIA

FACTORS INFLUENCING POPULATIONS OF *EUMARGARODES LAINGI* AND *PROMARGARODES* SPP. IN SUGARCANE AT BUNDABERG AND DERIVATION OF SEQUENTIAL SAMPLING PLANS

by

P W Walker and P G Allsopp

TE92002

BSES Publication
Technical Reports TE92002 **March 1992**

CONTENTS

Page No.

SUMMARY

INTRODUCTION 1

PART ONE - FACTORS INFLUENCING POPULATIONS

MATERIALS AND METHODS 2

RESULTS 3

- Soil type 3
- Crop cultivar and age 4
- Fallow factors 5
- Pesticide use 7
- Irrigation 8

DISCUSSION 8

PART TWO - SPATIAL DISTRIBUTION AND SEQUENTIAL SAMPLING PLANS

MATERIALS AND METHODS 11

- Statistical analysis 11
- Sampling plans 11
- Variance stabilisation 12

RESULTS AND DISCUSSION 13

- Sampling distributions 13
- Sampling plans 16
- Variance stabilisation 16

ACKNOWLEDGMENTS 19

REFERENCES 19

SUMMARY

A survey of the margarodids *Eumargarodes laingi* Jakubski and *Promargarodes* spp. in sugarcane was carried out at Bundaberg. *E. laingi* cyst numbers were influenced by soil type; *Promargarodes* spp. numbers did not differ between soil types, but there were differences between cultivars. There were more *E. laingi* and *Promargarodes* spp. cysts in older crops. The number of times the previous fallow was ploughed and rotary hoed influenced *E. laingi* numbers, but not *Promargarodes* spp. numbers. There were fewer *E. laingi* cysts following a long fallow, but crop rotations did not affect *E. laingi* numbers. There were trends to lower *Promargarodes* spp. numbers following a long fallow or a sugarcane-rockmelon rotation. Fields treated with chlorpyrifos (emulsifiable concentrate) had lower numbers of *E. laingi* than those treated with controlled-release chlorpyrifos; *Promargarodes* spp. numbers were not influenced by insecticide applications. Soil pH and electrical conductivity, ripping during fallow, type of irrigation, and nematicides used did not affect numbers of *E. laingi* or *Promargarodes* spp.

Sampling statistics were determined for cysts of both margarodid types. The Poisson distribution, Iwao's regression model and Taylor's power law were used to determine the relationship between mean and variance of counts of both types of margarodids from sugarcane crops of different ages. All models indicated that the cysts were aggregated in distribution. Taylor's power law generally gave better or equivalent fits to the population dispersion parameters than did Iwao's regression model. Relationships to determine sample sizes for fixed levels of precision and fixed-precision-level stop lines for sequential sampling were developed for each margarodid genus. There were functional relationships between the variance and mean of untransformed population counts for all margarodids and crop ages, and the suitability of three transformation functions is assessed.

INTRODUCTION

Four species of margarodids or ground pearls feed on roots of Queensland sugarcane: pink ground pearl *Eumargarodes laingi* Jakubski, *Promargarodes australis* Jakubski, *P. williamsi* Jakubski, and an undescribed species near *P. sinensis* Silvestri (P J Gullan unpublished data). In the Bundaberg area of southeastern Queensland, *E. laingi* is a serious pest of sugarcane (Dominiak *et al* 1989). During the 1990 season, *E. laingi* caused losses of 20 600 t of sugarcane, valued at $A0.9m (BSES unpublished data). *E. laingi* also occurs on grasses in Florida, Georgia and Alabama (Spink 1953). *Promargarodes* spp. are not considered to be serious pests of Australian sugarcane. However, *P. australis* is common and more widely distributed throughout Queensland than is *E. laingi* (Gullan unpublished data). In pot trials, Hitchcock (1965) reported a 21% decrease in millable cane when sugarcane stools were infested with 10 000 cysts of *P. australis*.

Adult females of *E. laingi* emerge over an extended period from September through February, with the main emergence in November and December (Hitchcock 1965). They lay about 600 eggs close to the soil surface, and these hatch in about two weeks. First instars move through the soil for about three weeks, and then commence feeding on a root. A test is secreted, covering the body except for the feeding tube, antennae and legs. After two months, the first instar moults and secretes a new test to form the cyst stage. Second instars have long feeding tubes, reduced antennae and no legs, and remain encysted for the rest of their development. They moult to adults within the cyst, from which the adult then escapes. *E. laingi* is univoltine, while *Promargarodes* spp. may take up to four years to complete development (Hitchcock 1965). *Promargarodes* spp. females are not found on the surface, and some remain in the cyst to oviposit.

Once they are established in a sugarcane field, control of margarodids is extremely difficult. All insecticides so far field-tested (except methyl bromide) have failed to adequately control nymphs or adults (Spink and Dogger 1961, Hitchcock 1965, Dominiak *et al* 1989). Fallowing fields and cultivation of infested areas while adults, eggs, and first instars are close to the surface have been promoted as useful control measures (Hitchcock 1965).

As part of on-going studies on margarodids, we surveyed sugarcane farms in the Bundaberg area for margarodids. We attempt to correlate margarodid numbers with sugarcane cultivar and age, soil type, and agronomic practices. From these correlations we formulate hypotheses which can be tested under more rigorous experimentation. We also report on the spatial distribution of *E. laingi* and *Promargarodes* spp. cysts and assess the suitability of two mathematical models to describe the distributions. We then develop relationships to determine sample sizes for fixed levels of precision and fixed-precision-level stop lines for sequential sampling. We also assess the usefulness of three transformations in standardising mean-variance relationships.

PART ONE
FACTORS INFLUENCING POPULATIONS

MATERIALS AND METHODS

Margarodids were sampled in 122 sugarcane fields on 11 farms in the Bundaberg area, southeastern Queensland, between May and August 1991. Populations were then in the cyst stage (Hitchcock 1965). Farms known to have an infestation of *E. laingi* were selected for the survey. On each farm, sugarcane fields were chosen at random for sampling and included a range of crop cultivars and ages.

In each field we took 5-20 soil samples depending on the size of the field. Each sample was 2.78 litres of soil, half dug from either side of a sugarcane stool to a depth of 14 cm. Samples were taken back to the laboratory for extraction of margarodids. Samples were washed individually and some of the organic material was floated off. After washing, large pieces of organic material were removed with a 4-mm sieve and *E. laingi* and *Promargarodes* spp. cysts collected in a 0.5-mm sieve. Cysts were then counted. We distinguished each genus by the colour and hardness of the cyst (Hitchcock 1965). No attempt was made to differentiate species of *Promargarodes* as this is difficult, requiring each individual to be slide mounted.

For each field we recorded sugarcane cultivar, crop age, length of previous fallow, previous crop rotation, method of fallow cultivation, planting time, and previous insecticide and nematicide use. A sample of soil was taken from the top 15 cm of each field and sand, silt, and clay composition determined using the mechanical analysis method of Piper (1942). Soils were grouped on these analyses using the triangular textural diagram (McDonald *et al* 1984, Fig. 13). Soil pH was determined with a TPS Ion Analyser after mixing 20 g of air-dried soil with 100 g of deionised water and shaking for 1 hour. Electrical conductivity was determined on the same mixture with a Philips PW9501 conductivity meter.

Data were analysed with one-way analysis of variance (ANOVA); pairwise comparisons were made with t-tests, modified where necessary for unequal variances. Before analysis, counts of *E. laingi* were transformed with $x^{0.128}$ and counts of *Promargarodes* spp. were transformed with $\ln(x+1)$. These are the most appropriate transformations to make variances independent of means and to normalise distributions of means (Part Two). The Wilcoxon signed rank test was used on untransformed data to test the dominance of any one species in collections. Spearman's rank correlation was used to correlate untransformed numbers of *E. laingi* and *Promargarodes* spp. with soil texture components, soil pH and soil electrical conductivity. All analyses used procedures from STATISTIX 3.1 (Analytical Software 1989).

RESULTS

We collected 16 766 *E. laingi* and 3 930 *Promargarodes* spp. from the 122 fields during sampling. All were in the cyst stage, except for one adult female *E. laingi* collected on 28th June. Numbers of *E. laingi* were greater than those of *Promargarodes* spp. at 78 sites and less at 43 sites ($P < 0.0001$). Numbers of the two species were significantly, but weakly, correlated across all sites ($r = 0.191$, $df = 120$, $P < 0.05$).

Soil type

There were significant differences in *E. laingi* numbers in fields on different soil types ($F = 2.17$, $df = 7{,}113$, $P = 0.041$) (Table 1). There were no significant ($P < 0.05$) correlations of *E. laingi* numbers with clay ($r = 0.02$), sand ($r = -0.01$), or silt ($r = 0.13$) contents of the soil. There were no significant differences in *Promargarodes* spp. numbers on different soil types ($F = 1.02$, $df = 7{,}113$, $P = 0.42$) (Table 1).

Table 1

Mean transformed number (SE) of *Eumargarodes laingi* and *Promargarodes* spp. in samples from different soil types

Soil type	n	*E. laingi*	*Promargarodes* spp.
Clay	58	1.15 (0.10)ab	2.33 (0.15)
Silty clay	32	1.37 (0.11)b	2.53 (0.19)
Silty clay loam	5	1.41 (0.18)ab	2.36 (0.57)
Clay loam	8	0.61 (0.30)ac	2.36 (0.50)
Sandy clay loam	1	0.20	0.58
Silty loam	2	1.28 (0.37)ab	3.33 (0.19)
Loam	4	0.31 (0.11)c	2.53 (0.63)
Sandy loam	4	0.98 (0.30)abc	1.17 (0.45)
Loamy sand	8	1.07 (0.12)ab	2.56 (0.27)

Means followed by different letters are different at $\alpha = 0.05$. Sandy clay loam was excluded from analyses.

There was no significant correlation between *E. laingi* numbers and soil pH ($r = 0.173$, $df = 120$, $P > 0.05$). However, there was a weak, but significant, negative correlation between *Promargarodes* spp. numbers and soil pH ($r = -0.189$, $df = 120$, $P < 0.05$). There was no significant correlation between soil electrical conductivity and *E. laingi* numbers ($r = -0.021$, $df = 120$, $P > 0.05$) or *Promargarodes* spp. numbers ($r = 0.105$, $df = 120$, $P > 0.05$).

Crop cultivar and age

There were no significant differences in *E. laingi* numbers amongst cultivars with more than one sample (F = 1.44, df = 9,108, P = 0.18) (Table 2). There were significant differences in *Promargarodes* spp. numbers on cultivars with more than one sample (F = 2.64, df = 9,108, P = 0.0084) (Table 2). Numbers were significantly (P < 0.05) lower on CP51-21 than on CP44-101, Q87, Q110, Q125, or Q144, and significantly (P < 0.05) lower on Q146 than on Q87 or Q144.

Table 2

Mean transformed number (SE) of *Eumargarodes laingi* and *Promargarodes* spp. in samples from under different cultivars of sugarcane

Cultivar	n	*E. laingi*	*Promargarodes* spp.
CP44-101	27	0.91 (0.15)	2.63 (0.20)ab
CP51-21	13	0.91 (0.16)	1.42 (0.27)c
H56-752	8	1.55 (0.24)	2.28 (0.48)abc
Q87	3	1.23 (0.62)	3.67 (0.81)a
Q99	1	1.03	2.01
Q110	19	1.33 (0.15)	2.79 (0.27)ab
Q111	1	0.84	2.46
Q125	4	1.23 (0.39)	2.71 (0.36)ab
Q137	1	0.00	3.30
Q140	10	1.60 (0.22)	2.08 (0.40)abc
Q141	17	1.13 (0.18)	2.21 (0.26)abc
Q144	5	1.03 (0.32)	3.05 (0.23)a
Q145	1	0.20	0.83
Q146	12	1.16 (0.13)	2.04 (0.22)bc

Means followed by different letters within columns are different at $\alpha = 0.05$. Cultivars Q99, Q111, Q137, and Q145 were excluded from analyses.

E. laingi numbers also varied significantly on crops of different ages (F = 4.70, df = 4,117, P = 0.0016) (Table 3). There were significantly (P < 0.05) fewer on plant and first-ratoon crops than on second- and third-ratoon crops. Numbers on crops older than third ratoon were much more variable than those on younger crops and were not significantly (P > 0.05) different. *Promargarodes* spp. numbers also varied significantly between crops of different ages (F = 8.96, df = 4,117, P < 0.0001) (Table 3). There were significantly (P < 0.05) fewer on plant and first-ratoon crops than on third-ratoon crops.

Table 3

Mean transformed number (SE) of *Eumargarodes laingi* and *Promargarodes* spp. in samples from sugarcane crops of different ages

Crop age	n	*E. laingi*	*Promargarodes* spp.
Plant	28	0.80 (0.11)b	2.12 (0.19)cd
First ratoon	26	0.99 (0.13)b	1.55 (0.22)d
Second ratoon	29	1.42 (0.14)a	2.47 (0.18)bc
Third ratoon	22	1.45 (0.11)a	3.01 (0.16)a
Fourth ratoon and older	17	1.05 (0.21)ab	3.05 (0.29)ab

Means followed by different letters within columns are different at $\alpha = 0.05$.

Fallow factors

E. laingi numbers were significantly lower (F = 4.09, df = 2,119, P = 0.019) where there had been a fallow of 10-14 months ($\bar{x}$ = 0.60, SE = 0.14, n = 11) than where the previous sugarcane crops had been ploughed-out and the new one had been replanted immediately ($\bar{x}$ = 1.09, SE = 0.14, n = 27), or where there had been a fallow of 5-7 months before replanting ($\bar{x}$ = 1.23, SE = 0.08, n = 84). There was a trend to lower *Promargarodes* spp. numbers (F = 2.60, df = 2,119, P = 0.077) following a long ratoon ($\bar{x}$ = 1.74, SE = 0.28, n = 11) compared to following a ploughout-replant ($\bar{x}$ = 2.65, SE = 0.21, n = 27), or a 5-7 months fallow ($\bar{x}$ = 2.37, SE = 0.12, n = 84).

To assess the effects of crops grown in rotation with sugarcane, we considered only the 95 fields with a break between sugarcane crops of at least five months. There were no significant differences in *E. laingi* numbers when successive crops of sugarcane were grown ($\bar{x}$ = 1.14, SE = 0.09, n = 68), or where rockmelons ($\bar{x}$ = 1.14, SE = 0.16, n = 20), or tomatoes ($\bar{x}$ = 1.34, SE = 0.28, n = 7) were grown after the previous sugarcane crop (F = 0.37, df = 2,95, P = 0.70). There was a trend to lower *Promargarodes* spp. numbers (F = 2.99, df = 2,95, P = 0.053) in fields which had a sugarcane-rockmelons-sugarcane rotation ($\bar{x}$ = 1.82, SE = 0.22, n = 20) rather than sugarcane-sugarcane ($\bar{x}$ = 2.38, SE = 0.14, n = 68) or sugarcane-tomatoes-sugarcane rotations ($\bar{x}$ = 2.87, SE = 0.50, n = 7).

There were significant differences in *E. laingi* numbers depending on the number of times the previous fallow was ploughed (F = 4.16, df = 4,117, P = 0.0036), although there were no systematic changes in numbers with ploughing frequency (Table 4). There was no significant effect on *Promargarodes* spp. numbers of the number of times the fallow was ploughed (F = 0.52, df = 4,117, P = 0.73) (Table 4).

Table 4

Mean transformed number (SE) of *Eumargarodes laingi* and *Promargarodes* spp. in samples from crops ploughed with different frequencies during previous fallow

Times ploughed	n	*E. laingi*	*Promargarodes* spp.
0	27	1.44 (0.11)b	2.36 (0.22)
1	26	1.07 (0.14)ac	2.54 (0.23)
2	19	1.16 (0.15)abc	2.56 (0.24)
3	35	0.82 (0.13)c	2.23 (0.20)
4	15	1.45 (0.16)ab	2.18 (0.27)

Means followed by different letters are different at $\alpha = 0.05$.

There were no significant differences in *E. laingi* numbers (F = 2.31, df = 3,118, P = 0.078) or in *Promargarodes* spp. numbers (F = 1.85, df = 3,118, P = 0.14) depending on the number of times the previous fallow was ripped (Table 5).

Table 5

Mean transformed number (SE) of *Eumargarodes laingi* and *Promargarodes* spp. in samples from crops ripped with different frequencies during previous fallow

Times ripped	n	*E. laingi*	*Promargarodes* spp.
0	32	1.07 (0.12)	2.64 (0.20)
1	67	1.17 (0.09)	2.38 (0.14)
2	8	0.67 (0.23)	1.60 (0.38)
3	15	1.45 (0.16)	2.23 (0.27)

There were significant differences in *E. laingi* numbers (F = 7.34, df = 3,118, P = 0.0002) depending on the number of times the previous fallow was rotary hoed. Numbers decreased with up to three rotary hoeings (Table 6). There were no significant differences in *Promargarodes* spp. numbers (F = 1.45, df = 3,118, P = 0.23) depending on the number of times the previous fallow was rotary hoed (Table 6).

Table 6

Mean transformed number (SE) of *Eumargarodes laingi* and *Promargarodes* spp. in samples from crops rotary hoed with different frequencies during previous fallow

Times hoed	n	*E. laingi*	*Promargarodes* spp.
1	28	1.54 (0.09)a	2.52 (0.18)
2	58	1.18 (0.09)b	2.41 (0.15)
3	28	0.80 (0.15)c	2.37 (0.23)
4	8	0.67 (0.23)c	1.60 (0.38)

Means followed by different letters are different at $\alpha = 0.05$.

Pesticide use

There were significant differences in *E. laingi* numbers depending on the type of insecticide applied at planting ($F = 5.99$, df = 3,118, $P = 0.0009$) (Table 7). Numbers were significantly ($P < 0.05$) higher where CR-chlorpyrifos (suSCon Blue) was applied than where only EC-chlorpyrifos (Lorsban) was applied; comparisons with untreated fields are difficult as only two fields received neither suSCon nor Lorsban. There were no significant differences in *Promargarodes* spp. numbers following different insecticide treatments ($F = 1.15$, df = 3,118, $P = 0.33$) (Table 7).

Table 7

Mean transformed number (SE) of *Eumargarodes laingi* and *Promargarodes* spp. in samples from crops treated with different insecticides at planting

Insecticide	n	*E. laingi*	*Promargarodes* spp.
Lorsban	76	0.95 (0.09)b	2.27 (0.13)
suSCon blue	40	1.50 (0.08)a	2.46 (0.17)
Lorsban + suSCon	4	1.29 (0.13)a	3.26 (0.52)
None	2	1.00 (0.40)ab	2.62 (0.37)

Means followed by different letters are different at $\alpha = 0.05$.

E. laingi numbers were significantly ($P < 0.05$) higher in fields with past applications of BHC alone or in combination with dieldrin than in fields with dieldrin alone or neither insecticide ($F = 5.16$, df = 3,118, $P = 0.0023$) (Table 8). Numbers of *Promargarodes* spp. were not affected by past applications of BHC or dieldrin ($F = 0.77$, df = 3,118, $P = 0.52$) (Table 8).

Table 8

Mean transformed number (SE) of *Eumargarodes laingi* and *Promargarodes* spp. in samples from crops in fields with past applications of BHC or dieldrin

Past insecticide use	n	*E. laingi*	*Promargarodes* spp.
BHC + dieldrin	96	1.24 (0.07)a	2.42 (0.12)
BHC alone	12	1.08 (0.16)a	2.22 (0.26)
Dieldrin alone	8	0.57 (0.17)b	2.53 (0.32)
None	6	0.39 (0.27)b	1.76 (0.57)

Means followed by different letters are different at $\alpha = 0.05$.

There were no significant differences in *E. laingi* numbers (t = 1.60, df = 120, P = 0.12) between fields treated with a nematicide in the previous 12 months ($\bar{x}$ = 1.37, SE = 0.14, n = 20) and those that were not treated ($\bar{x}$ = 1.11, SE = 0.07, n = 102), or in *Promargarodes* spp. numbers (t = 0.48, df = 120, P = 0.63) between nematicide-treated ($\bar{x}$ = 2.26, SE = 0.25, n = 20) and untreated ($\bar{x}$ = 2.39, SE = 0.11, n = 102) fields.

Irrigation

Sixteen fields received both overhead and furrow irrigation and were excluded from analysis. There were no significant differences in *E. laingi* numbers (t = 1.42, df = 72.4, P = 0.16) between overhead-irrigated ($\bar{x}$ = 1.15, SE = 0.08, n = 76) and furrow-irrigated ($\bar{x}$ = 1.33, SE = 0.10, n = 30) fields, or in *Promargarodes* spp. numbers (t = 0.53, df = 104, P = 0.60) between overhead-irrigated ($\bar{x}$ = 2.40, SE = 0.13, n = 76) and furrow-irrigated ($\bar{x}$ = 2.52, SE = 0.18, n = 30) fields.

DISCUSSION

Our results indicate that *E. laingi* numbers may be influenced by soil type, crop age, ploughing and rotary hoeing during fallows, fallow length, and insecticides used at planting. *Promargarodes* spp. numbers may be influenced by crop cultivar and age, fallow length, type of crop grown between successive sugarcane crops, and length of the fallow between successive sugarcane crops.

We detected some differences in numbers of *E. laingi* in crops on different soil types. Infested fields are mainly on clays and silty clays, known locally as red volcanics (Dominiak *et al* 1989), but our survey shows that *E. laingi* can survive in a wide range of soil types. There appears to be a trend to lower numbers in loamy soils, but this needs to be confirmed in more rigorous experiments. Spread of *E. laingi* to other soil types and districts represents a real danger as cysts can be transported in contaminated soil on farm machinery (Dominiak *et al* 1989). *E. laingi* numbers were not affected by soil pH or soil electrical conductivity. *Promargarodes* spp. numbers were unaffected by soil type; *P. australis* occurs in soils from heavy clays, through red volcanic loams, to podzols (Dominiak *et al* 1989). There was a weak correlation with low soil pH.

Past observations have suggested that sugarcane cultivars respond differently to margarodids (Hitchcock 1965, Dominiak *et al* 1989). We detected no effects of cultivar on numbers of *E. laingi*, but some effects on numbers of *Promargarodes* spp. However, the extent of cultivar effects may have been masked by early plough-out of susceptible cultivars. Dominiak *et al* (1989) observed that CP44-101 is quite susceptible to *E. laingi*, but in pot experiments *E. laingi* multiplied similarly under five sugarcane cultivars (unpublished data). This suggests that the tolerance shown by different sugarcane cultivars may be more important in determining resistance than are the effects of nonpreference or antibiosis of different cultivars.

Numbers of *E. laingi* generally increased with crop age up to third-ratoon crops. *E. laingi* is univoltine, with limited dispersal of adult females and first instars and a sessile second instar. Soil is disturbed little during a crop cycle, minimising mortality and induced dispersion. Hence, increasing numbers with crop age is consistent with a build-up, with breeding from those cysts carried over from the previous crop cycle. The apparent decrease in numbers after the third ratoon is presumably because heavily-infested fields are ploughed out by this stage. The trend in *Promargarodes* spp. numbers with crop age is similar, with fewer in plant and first-ratoon crops than in older crops. The mechanism is the same as for *E. laingi*, except the differences between crop ages are masked by the longer life cycle of *Promargarodes* spp.

Numbers of *E. laingi* were lower following long fallows, but were independent of the crop grown in the fallow. This is consistent with a field trial which showed that planting tomatoes did not reduce inter-row populations any more than did replanting with sugarcane or bare fallowing for five months during autumn-winter (Dominiak *et al* 1989). Longer fallows may be more effective in reducing numbers, as *E. laingi* is relatively susceptible to starvation (Hitchcock 1965). The annual life cycle of *E. laingi* also means that cultivation just before spring planting affects adults, eggs, and first instars; cultivation just before autumn planting would affect only cysts near the surface. There is also only a small amount of root material available on spring-planted crops for colonisation by nymphs in spring and early summer. There were trends to lower *Promargarodes* spp. numbers following a rotation with rockmelons or following a long fallow. These trends may reflect the survival of *Promargarodes* spp. cysts for up to three years without food and their longer life cycles (Hitchcock 1965).

We found that *E. laingi* numbers were affected by the number of times a field was ploughed or rotary-hoed, but not by the number of times it was ripped. Ploughing inverts soil, exposing cysts and remains of sugarcane plants to desiccation. Rotary hoeing is the most severe cultivation method, breaking up pieces of sugarcane plant and pulverising soil. Ripping is relatively gentle, merely disturbing soil structure. We detected no effects of cultivation on *Promargarodes* spp., perhaps reflecting the harder cysts of *Promargarodes* spp. and their ability to better survive periods of starvation (Hitchcock 1965).

We also showed that the type of insecticide applied at planting appeared to effect subsequent numbers of *E. laingi*. Fields treated with Lorsban had lower numbers than those treated with suSCon. The effect appears to be an increase in numbers following suSCon application rather than a decrease following application of Lorsban, although the low number of untreated fields makes this comparison difficult. SuSCon releases over three years and might have a continued effect on as yet unidentified predators and pathogens of *E. laingi*. Lorsban remains effective for only a short time, perhaps not affecting natural enemies or allowing them to recolonise quickly. Alternatively, fields prone to attack by sugarcane-infesting scarabs, and hence treated with suSCon, may have crops under stress. Stress is known to induce changes in the nutritional quality of sugarcane plants (Wiggins and Williams 1955, Fennah 1969), and this may make them more suitable for pest growth. The apparent effect of BHC may be similar to that of suSCon; BHC has a long residual life and is ineffective against *E. laingi* (Dominiak *et al* 1989). Past application of dieldrin appeared to have no effect on *E. laingi* numbers, consistent with it being ineffective against *E. laingi* (Dominiak *et al* 1989), but inconsistent with its long residual life. Numbers of *Promargarodes* spp. were not affected by past insecticide application.

The absence of any apparent effect of nematicides on both types of margarodid is in agreement with field tests of aldicarb to 15 kg (AI)/ha which did not significantly reduce counts of *E. laingi* cysts in subsequent sugarcane crops (Dominiak *et al* 1989).

There were no fields in the study which were not irrigated but the method of irrigation had no effect on numbers of *E. laingi* or *Promargarodes* spp. However, furrow irrigation can move adults down slopes (Dominiak *et al* 1989) to infest new areas; overhead irrigation presumably limits such movement.

Our findings indicate that further investigation of crop resistance (especially tolerance), effects of long fallowing, ploughing, rotary hoeing, and planting date, and long-term effects of suSCon would be areas for useful research aimed at minimising numbers of *E. laingi*. The apparent reasonable survival of *E. laingi* in a wide range of soil types raises concern about the species' distribution being widened through dispersal on contaminated machinery. *Promargarodes* spp. numbers are influenced by crop cultivar and fallow conditions. Although *Promargarodes* spp. are not major pests, experiments on *E. laingi* should also consider the effects on *Promargarodes* spp. to minimise possible increases in *Promargarodes* spp. numbers and consequent effects on crops. Crop age is an important factor affecting population levels of both margarodid types; heavily-infested fields must be ploughed out and fallowed for as long as possible.

PART TWO
SPATIAL DISTRIBUTIONS AND SEQUENTIAL SAMPLING PLANS

MATERIALS AND METHODS

Statistical analysis

Each set of samples collected in the field study was classified according to crop age. Data from fourth- to eighth-ratoon crops were considered together, as there were few samples from any one of these crop ages. Means ($\bar{x}$) and variances (s^2) were calculated for *E. laingi* and *Promargarodes* spp. in each set of samples. Sets of samples with a mean density of zero and duplicate mean-variance combinations were excluded from the analyses. The means and variances were used to test if the counts conformed to the Poisson (random) distribution (Southwood 1978). Dispersion indices were calculated for each margarodid genus using Iwao's patchiness regression (Iwao 1968) and Taylor's power law (Taylor 1961).

If a population follows the Poisson distribution, the mean density will equal the variance, so that the departures of $s^2/\bar{x}$ from unity will be a measure of the departure from the Poisson distribution. We tested this by the index (I_D), where

$$I_D = [s^2(n-1)]/\bar{x}$$

where n is the number of units comprising a sample. The significance of I_D was tested as χ^2 with n-1 degrees of freedom (Southwood 1978).

Iwao's patchiness regression uses the linear regression of Lloyd's (1967) mean crowding index ($M = \bar{x} + [(s^2/\bar{x}) - 1]$) on the mean density through the model $M = \alpha + \beta\bar{x}$, where α and β are least-squares parameters. The slope β, the density-contagiousness coefficient, supposedly describes how individuals distribute themselves.

Taylor's power law relates variance to mean density through $s^2 = a\bar{x}^b$, where a is suggested to be a sampling factor, and b is suggested to be an index of aggregation (Taylor 1961). We obtained least-squares estimates of a and b by regressing s^2 on $\bar{x}$ using the logarithmic transformation of the power law.

We tested differences in slopes and intercepts with the t-test.

Sampling plans

We developed relationships to determine sample sizes for fixed levels of precision by substituting Taylor's variance-mean relationship into the usual expression for the standard error of the mean and rearranging:

$$n = \frac{a.\bar{x}^{(b-2)}}{p^2}$$

where n is the sample size and p is the required level of precision expressed as a proportion of the mean. We used two values of p, 0.10 and 0.25; the latter allows detection of doubling or halving of sample means (Southwood 1978), whereas the former would be useful in detecting smaller changes in population monitoring. Lines were calculated for *E. laingi* using the Taylor's parameters for the combined data (all crop ages) and for *Promargarodes* spp. using the combined data for plant to second-ratoon crops.

Fixed-precision-level stop lines for sequential sampling were calculated using Green's (1970) formula:

$$\ln T_n = \frac{\ln(p^2/a)}{b-2} + \frac{b-2}{b-1}.\ln n$$

where T_n is the cumulative number of cysts, p is the precision level, and n is the number of samples. We considered a precision level of 0.25, and calculated species-crop age lines as above.

Variance stabilisation

Assessing sampling variance in the analysis of variance model presupposes a normal distribution in which the mean and variance are independent. If the mean and variance are functionally related, it is essential to transform the data to stabilise the variance. We tested the adequacy of the square-root transformation (sqrt(x+0.5)), the ln(x+1) transformation and Healy and Taylor's (1962) transformation ($x^{1-b/2}$) based on Taylor's power law for the combined data for *E. laingi* and for the three crop-age combinations for *Promargarodes* spp. The necessity for transformation and the adequacy of the transformation functions were tested with simple correlation.

All calculations used STATISTIX 3.1 (Analytical Software 1989).

RESULTS AND DISCUSSION

Sampling distributions

The number of data sets with a mean density >0 and the ranges of sample mean densities for each margarodid genus on crops of different ages are given in Table 9.

In the test of the Poisson distribution, 95 of the 111 data sets of *E. laingi*, and 102 of the 121 data sets of *Promargarodes* spp. had variance-mean ratios significantly different ($P < 0.05$) from 1. These data indicate that the distribution of cysts was clumped in the majority of samples and random in only a few.

Table 9

Range of means and sample sizes of *E. laingi* and *Promargarodes* spp. cysts from sugarcane of different crop ages

Crop age	*E. laingi*		*Promargarodes* spp.	
	No. samples with $\bar{x}>0$	Range of $\bar{x}$	No. samples with $\bar{x}>0$	Range of $\bar{x}$
Plant crop	25	0.2-192.4	28	0.2-100.5
First ratoon	22	0.2-211.2	26	0.2-259.2
Second ratoon	28	0.2-851.8	29	0.1-425.5
Third ratoon	22	2.0-496.5	22	0.7- 94.3
Fourth-eighth ratoon	14	0.1-2 741.0	16	1.1-184.2

For *E. laingi* counts, Iwao's regression model gave significant linear regressions ($P < 0.01$) for all crop ages (Table 10). The R^2 for first-ratoon crops is low at 0.36, but it improves for other crop ages (0.70-0.98) (Table 10). All regression slopes are >1, indicating aggregated distributions; there are no significant differences ($P > 0.05$) between regression slopes. All values of α are positive, suggesting that within-row populations exist as groups of cysts; α for plant crops is significantly ($P < 0.05$) lower than for older crops (Table 10).

Table 10

Regression statistics for Iwao's regression for *E. laingi* and *Promargarodes* spp. cysts from sugarcane of different crop ages

Crop age	α (SE)	β (SE)	F	R^2
E laingi				
Plant crop	15.03 (11.28)a	1.71 (0.20)a	74.4	0.76
First ratoon	75.22 (40.87)b	1.71 (0.51)a	11.1	0.36
Second ratoon	99.29 (66.22)b	1.46 (0.19)a	59.1	0.70
Third ratoon	89.96 (46.97)b	1.73 (0.20)a	72.2	0.78
Fourth-eighth ratoon	76.31 (39.21)b	1.45 (0.06)a	632.8	0.98
Promargarodes spp.				
Plant cane	-15.36 (7.08)a	2.53 (0.19)a	173.6	0.87
First ratoon	-4.42 (3.23)a	1.96 (0.06)b	1 238.0	0.98
Second ratoon	4.53 (12.00)a	2.29 (0.14)a	254.7	0.90
Third ratoon	-7.34 (26.16)a	3.07 (0.70)ac	19.4	0.49
Fourth-eighth ratoon	-15.81 (9.74)a	2.95 (0.13)c	510.3	0.97

Values within each column and within species followed by the same letter are not significantly different ($P > 0.05$).

For *Promargarodes* spp. counts, Iwao's regression model gave significant linear regressions ($P < 0.001$) for all crop ages (Table 10). The R^2 is generally higher than that for *E. laingi*, varying from 0.49 for third-ratoon crops to 0.98 for first-ratoon crops (Table 10). Values of β are also higher (1.96-3.07), indicating that the distribution of *Promargarodes* spp. cysts is more aggregated than for *E. laingi* cysts. The slope for first-ratoon crops is significantly lower ($P < 0.05$) than that for all other crop ages, and the slope for fourth-eighth ratoon crops is significantly greater ($P < 0.05$) than that for other crop ages except third-ratoon crops. This suggests a general increase in aggregation of *Promargarodes* spp. cysts with increasing crop age. Except for second-ratoon crops, values of α are negative and are not significantly different from each other ($P > 0.05$).

For *E. laingi* counts, Taylor's power law gave significant linear regressions ($P < 0.001$) for all crop ages (Table 11). The values of R^2 are generally higher (≥ 0.92) than those for Iwao's regression model, indicating that Taylor's power law is a better descriptor of the spatial distribution. There are no significant differences ($P > 0.05$) between slopes. All values are >1, indicating an aggregated distribution, and fall within the range of $1.4 < b < 2.0$, which Ruesink (1980) proposed as being typical for field crop pests. The regression intercepts are all positive and are not significantly different from each other ($P > 0.05$, Table 11). Given that the regression slopes and intercepts are similar for all crop ages, all data were combined and an overall regression calculated (Table 11).

For *Promargarodes* spp. counts, Taylor's power law gave significant linear regressions ($P < 0.001$) for all crop ages (Table 11). The generally higher values of R^2 (≥ 0.83) indicate that this model is a better descriptor of the spatial distribution of the cysts than is Iwao's regression model (Table 11). Values of b are >1, and they increase in older crops, suggesting that the distributions of *Promargarodes* spp. are more aggregated in older cane fields. There are significant differences among slopes ($P < 0.05$), increasing from 1.68 for first-ratoon crops to 2.10 for fourth-eighth ratoon crops. Regression intercepts are positive, except for fourth-eighth ratoon crops. As there were no differences between slopes and intercepts for plant, first-ratoon and second-ratoon crops, data for these crop ages were combined and an overall regression calculated (Table 11).

Two other models, based on the negative binomial equation and the Poisson distribution, have been proposed for the variance-mean relationship (Southwood 1978). Neither are appropriate to the distribution of margarodids in sugarcane fields. The good fit of Taylor's power law shows that the negative binomial model is not applicable; a Taylor-type relationship between mean and variance implies that the k of the negative binomial is not constant (Taylor *et al* 1979).

Table 11

Regression statistics for Taylor's power law for *E. laingi* and *Promargarodes* spp. cysts from sugarcane of different crop ages

Crop age	ln a (SE)	b (SE)	F	R^2
E laingi				
Plant cane	1.09 (0.17)a	1.73 (0.06)a	780.8	0.97
First ratoon	1.36 (0.40)a	1.78 (0.11)a	279.8	0.93
Second ratoon	1.39 (0.35)a	1.69 (0.07)a	573.6	0.95
Third ratoon	1.96 (0.51)a	1.67 (0.11)a	236.9	0.92
Fourth-eighth ratoon	1.46 (0.34)a	1.73 (0.08)a	483.7	0.98
Combined crops	1.32 (0.14)	1.74 (0.03)	2 788.0	0.96
Promargarodes spp.				
Plant cane	0.19 (0.49)ab	1.78 (0.16)ab	125.2	0.83
First ratoon	0.80 (0.21)a	1.68 (0.07)a	533.1	0.96
Second ratoon	0.54 (0.33)ab	1.73 (0.11)ab	234.3	0.90
Third ratoon	0.52 (0.39)b	1.85 (0.14)b	188.2	0.90
Fourth-eighth ratoon	-0.13 (0.36)c	2.10 (0.10)c	433.4	0.97
Combined plant to second ratoon	0.48 (0.16)	1.79 (0.05)	1 129.0	0.91

Values within each column and within species followed by the same letter are not significantly different ($P > 0.05$).

The Poisson distribution is a peculiar case of the general distribution which occurs when Taylor's a = b = 1.

Sampling plans

Generated plans to estimate samples sizes for *E. laingi* cysts in all crops and *Promargarodes* spp. cysts in plant to second-ratoon crops with precisions of 0.1 and 0.25 of the mean are given in Figure 1. At the same cyst density and precision level, more samples are required to estimate *E. laingi* cyst numbers than to estimate *Promargarodes* spp. numbers. This may be somewhat counteracted by the generally lower number of *Promargarodes* spp. cysts in sugarcane fields (unpublished data). Separate plans are needed to estimate sample sizes of *Promargarodes* spp. cysts in third-ratoon crops, and fourth-eighth ratoon crops.

Figure 2 gives generated fixed-precision-level stop lines for the same combinations of margarodid type and crop age with a precision level of 0.25. Stop lines can not be used directly in the field as samples must be processed in the laboratory to determine the number of cysts present. However, samples can be processed until the cumulative number of cysts crosses the stop line for the desired level of precision.

Variance stabilisation

For both *E. laingi* and *Promargarodes* spp., the variances and means of the untransformed cyst-count data were correlated for all crop ages (Table 12), indicating the need to transform the data for parametric statistical analysis. For *E. laingi*, the Healy and Taylor (1962) transformation gave the lowest correlations; five of the six crop-age categories had correlations that were not significantly ($P > 0.05$) different from zero. The failure of the log and square-root transformations is not surprising; the log transformation is the most appropriate transformation for data obeying Taylor's power law when b = 2 and the square-root transformation is most appropriate when Taylor's b = 1 (Healy and Taylor 1962). For *Promargarodes* spp., the $\ln(x+1)$ transformation was the most suitable, with four of the six crop-age categories having correlations that were not significantly ($P > 0.05$) different from zero. This reflects the higher values of Taylor's b. The appropriate transformation should be assessed in each case, or alternatively, the data should be analysed using non-parametric statistical tests which do not assume independence of variances and means or that the distribution of data is normal.

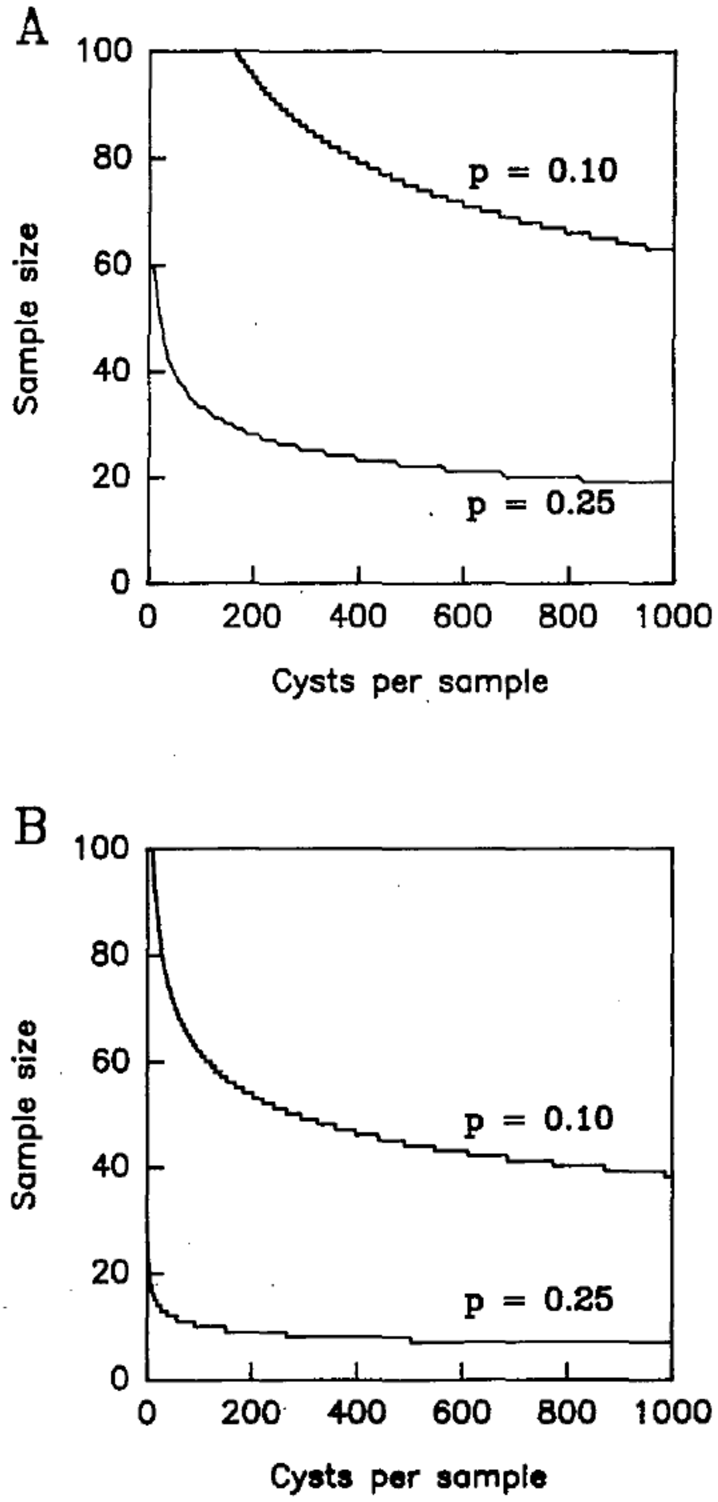

Fig. 1 Numbers of samples required to estimate populations of (A) *E. laingi* cysts in all crops and (B) *Promargarodes* spp. cysts in plant to second-ratoon crops of sugarcane with precisions of 0.1 and 0.25 (precision = 1 SE/mean).

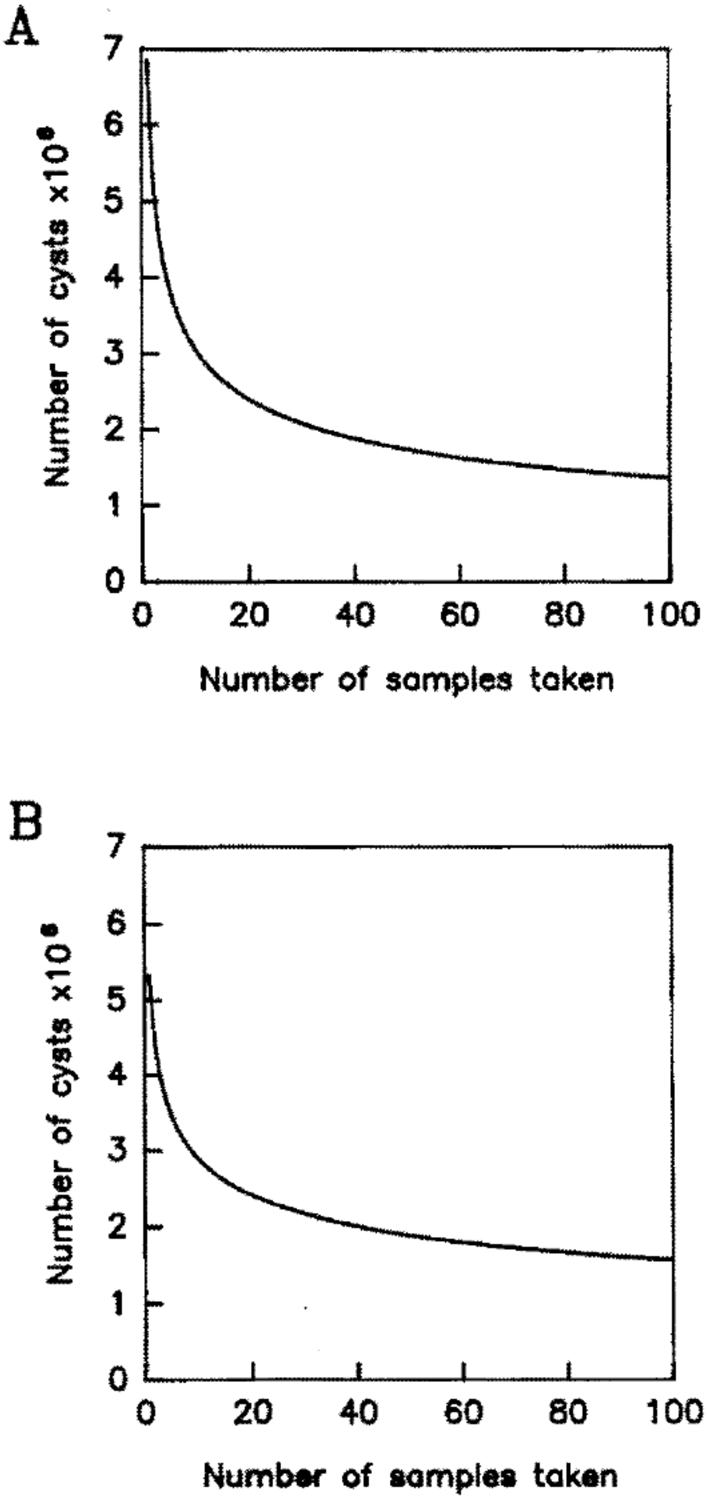

Fig. 2 Fixed-precision-level stop lines for sequential sampling of (A) *E. laingi* cysts in all crops and (B) *Promargarodes* spp. cysts in plant to second-ratoon crops of sugarcane with a precision of 0.25 (precision = 1 SE/mean).

Table 12

Correlation coefficients for the variance-mean relationship of original and transformed counts of *E. laingi* and *Promargarodes* spp. cysts from sugarcane of different crop ages

Crop age	Original counts	Transformation		
		Healy and Taylor	ln(x+1)	sqrt(x+0.5)
		E laingi		
Plant cane	0.93	0.18*	0.60	0.94
First ratoon	0.56	-0.07*	0.23*	0.79
Second ratoon	0.74	-0.28*	0.05*	0.91
Third ratoon	0.76	-0.61	-0.12*	0.81
Fourth-eighth ratoon	0.99	-0.08*	0.22*	1.00
Combined crops	0.89	-0.12*	0.23	0.98
		Promargarodes spp.		
Plant cane	0.84	-0.81	0.54	1.00
First ratoon	0.94	-0.37*	0.37*	0.98
Second ratoon	0.98	-0.52	0.34*	0.97
Third ratoon	0.57	-0.23*	-0.06*	0.80
Fourth-eighth ratoon	0.94	-0.72	-0.19*	0.99
Combined	0.90	-0.58	0.21	0.95

* Coefficients are not significantly ($P > 0.05$) different from zero.

ACKNOWLEDGMENTS

We thank Mike Turner and Col Spence (Millaquin-Qunaba Cane Protection and Productivity Board) and Keith Townsend (Fairymead Cane Protection and Productivity Board) for help with sampling, Norm McGill, Peter Samson, Keith Chandler, Bernie Dominiak, Trevor Willcox, and Joanne Stringer for useful discussion, and participating Bundaberg sugarcane growers for access to their fields and crop histories.

REFERENCES

Analytical Software (1989). Statistix 3.1. An interactive statistical analysis program for microcomputers. Analytical Software, St. Paul, Minn.

Dominiak, B C, Allsopp, P G and Corrie, K D (1989). The pink ground pearl *Eumargarodes laingi* Jakubski: past, present and future. Proc. Aust. Soc. Sugar Cane Technol., 11:110-115.

Fennah, R G (1969). Damage to sugar cane by Fulgoroidea and related insects in relation to the metabolic state of the host plant, pp. 367-389. In J R Williams, J R Metcalfe, R W Mungomery and R Mathes [eds], Pests of sugar cane. Elsevier, Amsterdam.

Green, R H (1970). On fixed precision level sequential sampling. Res. Popul. Ecol., 12:249-251.

Healy, M J R and Taylor, L R (1962). Tables for power-law transformations. Biometrika, 49:557-559.

Hitchcock, B E (1965). Investigations on earth pearls in Queensland cane fields. Proc. Int. Soc. Sugar Cane Technol., 12:1460-1465.

Iwao, S (1968). A new regression method for analyzing the aggregation pattern of animal populations. Res. Popul. Ecol., 10:1-20.

Lloyd, M (1967). Mean crowding. J. Anim. Ecol., 36:1-30.

McDonald, R C, Isbell, R F, Speight, J G, Walker J and Hopkins, M S (1984). Australian soil and land survey field handbook. Inkata Press, Melbourne, Australia.

Piper, C S (1942). Soil and plant analysis. University of Adelaide, Adelaide, Australia.

Ruesink, W G (1980). Sequential sampling plans for soybean arthropods, pp. 61-78. In Kogan, M and D C Herzog [eds]. Sampling methods in soybean entomology. Springer, New York.

Southwood, T R E (1978). Ecological methods with particular reference to the study of insect populations, 2nd ed. Chapman and Hall, London.

Spink, W T (1953). The biology and control of the ground pearl *Eumargarodes laingi* Jak. with some biological notes on *Margarodes meridionalis* Morr. (Homoptera: Coccoidea - Margarodidae). PhD dissertation, North Carolina State College.

Spink, W T and Dogger, J R (1961). Chemical control of the ground pearl, *Eumargarodes laingi*. J. Econ. Entomol., 54:423-424.

Taylor, L R (1961). Aggregation, variance and the mean. Nature (Lond.), 189:732-735.

Taylor, L R, Woiwod, I P and Perry, J N (1979). The negative binomial as a dynamic ecological model for aggregation, and the density dependence of k. J. Anim. Ecol., 48:289-304.

Wiggins, L F and Williams, J H (1955). Amino acid content of West Indies sugar cane. J. Agric. Food Chem., 3:341-345.