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The Influence of Seed Harvesting Ants in Annual Ryegrass Pastures and Their Possible Effects on the Epidemiology of Ryegrass Toxicity

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THE INFLUENCE OF SEED HARVESTING ANTS IN ANNUAL RYEGRASS PASTURES AND THEIR POSSIBLE EFFECTS ON THE EPIDEMIOLOGY OF RYEGRASS TOXICITY

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ABSTRACT

In the disease known as annual ryegrass toxicity, galls induced by a nematode, **Anguina agrostis**, replace seeds in ryegrass (**Lolium rigidum**) plants. Often these galls are colonised by a bacterium, **Corynebacterium rathayi**, which makes them toxic and frequently fatal to grazing animals. The ecology of ryegrass pasture ants was studied to determine their role in the epidemiology of this disease. Pitfall traps run over one year in ryegrass pastures revealed nineteen species of ants, all of which foraged maximally between December-March; the period corresponding to that when most seeds and galls are shed.

Artificial depots of ryegrass seeds, placed out in pastures, indicated that 22-30% of seeds were removed within 24 hours. The principal ant species taking seed, presented in decreasing order of importance, were **Pheidole** sp. J.D.M. 155, **Pheidole** sp. J.D.M. 37, **Melophorus** sp. 1 (ANIC) and **Rhytidoponera inornata.** Seed/bacterial gall/nematode gall choice experiments indicated that ants were unselective harvesters of these items. Most seeds were stored around nest entrances, although lesser amounts were retained beneath the soil. Certain species were more prone to store seed than others.

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INTRODUCTION

It has been established that many Australian ants are active seed takers. This has created problems in the rehabilitation of mine pits (Majer, 1980) and in the establishment of mixed pastures (McGowan, 1969). Harvester and decorator ants remove large quantities of seed as food, or for use as building materials.

Studies on seed harvesting ants have looked at seed harvesting in native forests (Majer, 1980) and seed selection of harvester ants in pastures (Mott and McKeon, 1977). However, little work has been done on seed harvesting by ants in grasslands in Western Australia. As a result, little is known about the overall capacity of ants to remove seed from these areas.

Seed removal by ants from pastures is particularly relevant to South Australia (McIntosh et al., 1967) and Western Australia (Gwyn and Hadlow, 1971) where annual ryegrass (**Lolium rigidum**) may be the dominant pasture component. It is in these areas that the livestock disease known as annual ryegrass toxicity occurs. Here, galls induced by a nematode, **Anguina agrostis** replace seeds of ryegrass plants. Sometimes the nematodes may introduce a bacterium, **Corynebacterium rathayi**, into the galls which subsequently become toxic to grazing animals. Data collected so far, indicates that the toxic agent of parasitized annual ryegrass is produced either by the plant in response to C. **rathayi** invasion or by the bacterium itself using a specific plant substrate (Stynes et al., 1979).

Ryegrass toxicity has, so far, only been found over limited areas in South Australia (Fisher et al., 1979) and Western Australia (Berry and Wise, 1975). However, where ryegrass is the dominant component of pastures, the risk of spread poses a serious threat to other cereal-growing/grazing areas in Australia.

Seed harvesting by ants may play an important role in the ecology of this disease as ants have been observed removing large quantities of seed from Western Australian pastures (Stynes unpublished data). Ants may therefore reduce the ryegrass density and remove infected galls from paddocks. Stynes and Wise (1980) found that the incidence of this disease increased in the year following cropping although the risk of stock loss fell in the second year and was negligible after three or more years of continuous pasture. Since cultivation may destroy or damage ant colonies, and as heavy summer stubble left after grain harvesting may hinder ant activity, it is possible that seed taking ants may play some role in the sequence of annual ryegrass toxicity outbreak and decline.

This investigation had four main aims: (1) to relate the seasonality of ant foraging to times of seed/gall production, (2) to establish which ants are important seed harvesters, (3) to determine the relative abundance and capacity of each species to remove seed and (4) to investigate the seed/gall preferences of the major seed harvesting ants.

METHODS

The field work was conducted at two farms ("Butterworth" and "Quartermaine") near Katanning (33°39'S, 117°34'E), 260 km south-east of Perth, where the climate is warm mediterranean and annual rainfall averages 487 mm.

All ants mentioned in this study have been sorted to species level. Specific names were given where possible. When these were unavailable, they were either coded with Western Australian Institute of Technology (J.D.M.) code numbers or, if voucher specimens are deposited there, with Australian National Insect Collection (ANIC) codes.

Phenology of Galls and Ants

The seasonality of gall drop in the study area was determined by B.A. Stynes (unpublished data) at the Butterworth site in 1979/80. Here, four blocks measuring 25m x 1.5m were subdivided into twenty plots. Commencing in mid-November, 1979, one plot per block was selected at random and material gathered using a 1m x 0.5m quadrat. The plant material from the quadrat was hand harvested and the seeds and galls on the ground gathered using a vacuum cleaner. Harvesting continued at weekly intervals until the end of March, 1980. Daily temperatures and rainfall were also recorded for this period. These data provided estimates of the number of galls on the ground, galls in plant flower heads, and the total galls present.

The field work on the phenology of the ants was conducted by B.A. Stynes and J.D. Majer at the Quartermaine farm, using pitfall traps, in 1979/80. These traps were Pyrex test tubes (length 15cm, internal diameter 1.8cm) sunk vertically at ground level within P.V.C. sleeves as described by Majer (1978). Each test tube contained a 3ml mixture of alcohol/glycerol preservative (70/30 v/v). One hundred and sixty-four traps were established within replicated plots representing grazed/ungrazed and/or recently cultivated/uncultivated treatments. Following a one-week settling-in period traps were run for 7 day periods per month between July, 1979-August, 1980. The collections were then hand sorted and identified.

Nest Density

Once the major seed taking species were known, their relative abundance was determined by mapping the nests in the 37 m x 22 m Quartermaine plot with a 5 metre boundary included to eliminate statistical edge effects. A visual estimate was also made of slope and grass density.

Spatial Distribution

The method employed to assess intra- and inter-specific nest association was the modification used by Briese and Macauley (1977) of the nearest-neighbour analysis devised by Clark and Evans (1954). Here, the average distances between ant species nests in the 45 by 30 m plot were calculated. Using these distances, a 37m by 22m boundary was established such that, the statistical-edge effect was eliminated. Only nests inside this boundary were used for establishing population density (p), but all nests were used when determining intra- or inter-specific interaction (i.e. nearest-neighbour nest measurements).

For intraspecific pattern analysis, the mean distance from individual colonies to their nearest neighbour (rA) was tested against the mean distance of an infinitely large, randomly distributed population of the same density (rE). This value can be found by the equation:

$$rE = \frac{1}{2\sqrt{p}}$$

where p is the nest density and was calculated from the number of nests (X) per unit area (Y) of the study site, and was found by the equation:

$$p = \frac{Xn-1}{Y\ m^2}$$

The ratio of observed to expected mean distances (R) was found using the equation:

$$R = \frac{rA}{rE}$$

This provides a measure of the distribution pattern. R ranges from 0 when there is maximum aggregation to 2.149 when the distribution is completely regular. A value of about 1 indicates randomness.

The significance of departure from randomness was tested by determing the standard variate

$$C = \frac{rA - rE}{\sigma rE}$$

where σrE is the standard error of the mean distance in the theoretical random population. OrE is found by:

$$\sigma rE = \frac{0.26136}{\sqrt{\text{No. of distance measurements X density}}}$$

Due to fairly small sample sizes, the levels of significance were determined using the Pearson III distribution, tables for which are given by Pearson and Hartley (1966). This method was utilised rather than the normal t-test because the distribution of nearest-neighbour distances in a randomly dispersed set of points becomes skewed when the number is small (Clark and Evans, 1954).

To determine interspecific pattern the above method was slightly modified. The observed mean distance (rA) became the average distance between colonies of species A (the species with the lesser number of colonies) and its nearest neighbour of species B. The expected mean distance (rE) was calculated from a theoretical random population of density equal to the combined densities of species A and B. However, when calculating OrE, only the density of species A was used for the determination of significance of departure from randomness. This method can detect interspecific spacing effects even if the populations considered have a relatively homogeneous distribution (Briese and Macauley, 1977).

Seed Taking

To determine the seed taking ants, and also their relative capacity to remove seed, forty seed depots (20 x 15 cm) were placed out in the 45 m by 30 m Quartermaine plot in an eight by five grid, spaced 5 metres apart. Forty ryegrass seeds were placed on each depot in the early morning, and at 2 hour intervals, for a 24 hour period, a count of the number of seeds removed was made at each depot. The soil temperature, ambient temperature, and relative humidity were also recorded at 2 hour intervals. Any ants observed taking seed were collected, preserved for later identification and the time of day recorded.

The 24 hour seed taking trial was conducted at the Butterworth site, in December 1980, to determine the general trend of seed taking. During this experiment wind removal of seed was a problem; often all the ryegrass seeds were blown from a depot. Where this occurred seed was replenished to the previous 2 hour count for that seed depot. The 24 hour seed taking trial was repeated at the Quartermaine plot in January and February 1981. In February the method differed in that twenty seeds, twenty nematode galls and twenty bacterial galls were used to establish whether the ants displayed any "seed" preferences.

A second method of establishing seed taking was also employed. A 25 x 25 x 25 cm soil core was taken around five nests of the four most common seed harvesters and the seed content analysed. Five unnested control soil cores were also removed. Seeds and galls were removed from the nests using a combination of sieving and flotation. The nest midden (crater) was treated separately from the below-ground nest. After being oven dried, whole "seed" was hand sorted, identified and counted as nematode galls, bacterial galls, or seed.

RESULTS

Seasonality and Occurrence of Ants in Pastures

The monthly rainfall and the mean monthly maximum and minimum temperatures recorded during the study periods are shown in Figures 1 and 5.

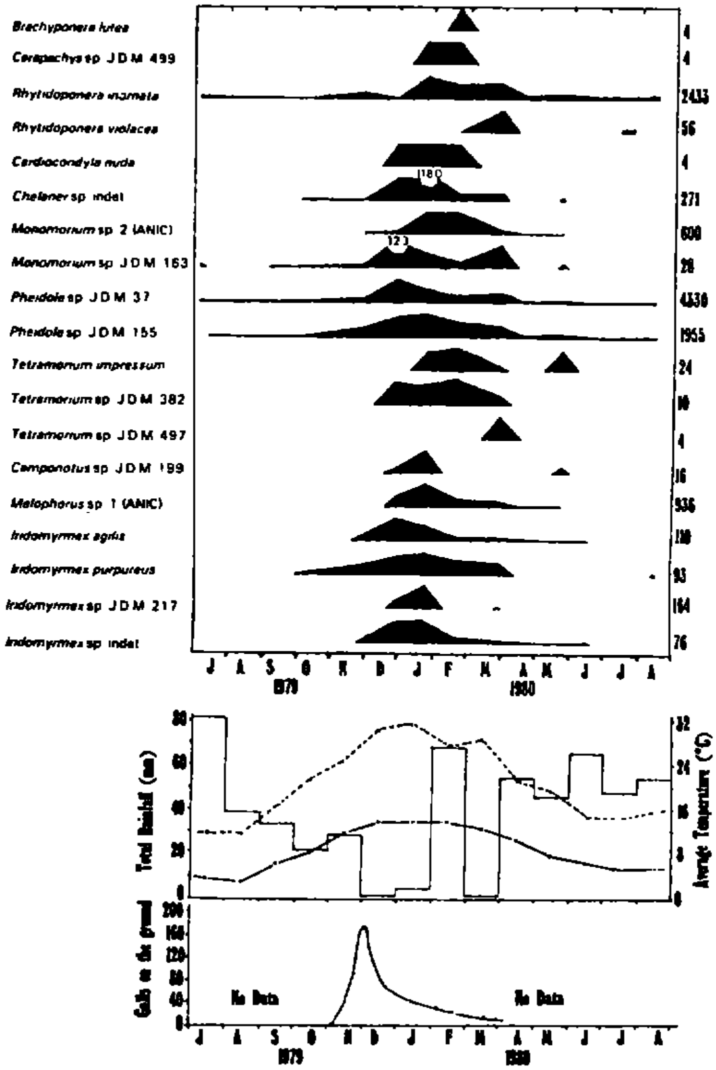

FIGURE 1

TOTAL ANTS PER 1148 TRAP NIGHTS OBTAINED AT THE QUARTERMAINE PLOT BETWEEN JULY 1979-AUGUST 1980. EXCEPT WHERE INDICATED THE NUMBER TO THE RIGHT OF EACH GRAPH IS THE MAXIMUM MONTHLY CATCH AND THE DATA ARE SCALED WITH RESPECT TO THIS. THE CORRESPONDING CLIMATIC DATA FOR KATANNING ARE ALSO SHOWN AS ARE THE NUMBER OF GALLS PER 0.5 M2 QUADRAT. LEGEND FOR CLIMATIC DATA: x---x, MEAN MAXIMUM TEMPERATURE; ●___●, MEAN MINIMUM TEMPERATURE; HISTOGRAM, TOTAL MONTHLY RAINFALL.

Nineteen species of ants from eleven genera were found in the Quartermaine Farm plot (Figure 1). No differences were observed in ant numbers in the grazed/ungrazed or recently cultivated/uncultivated plots (Majer, unpublished data) so the data were combined to investigate ant seasonality. All species were strongly seasonal in their activity and maximum foraging occurred between late-December and March. This corresponded with the warmest and driest part of the year. Not surprisingly it also corresponded to the period when seeds and galls were naturally shed. Figure 1 shows the numbers of galls collected at weekly intervals from the 1 x 0.5 m quadrats at the Butterworth Farm (Stynes unpublished data). On-ground galls reached a peak in early-December and density gradually declined until April.

Ant Distribution and Density

One hundred and fifty-three ant nests of four species were found in the Quartermaine area. This represents an overall nest density of 1133 nests per hectare. Figure 2 shows the distribution of these nests. The species mapped were **Pheidole** sp. J.D.M. 155 (93 nests), **Pheidole** sp. J.D.M. 437 (19 nests), **Melophorus** sp. 1 (ANIC) (21 nests) and **Rhytidoponera inornata** (20 nests). Other species were undoubtedly present but nests were insufficiently common or too inconspicuous to observe. Down slope and grass density cover of the Quartermaine plot are also shown in Figure 2.

Table 1 shows the results of the nearest neighbour analysis. All four species exhibited statistically significant aggregated distribution patterns. Reasons for these patterns are not yet known although **Melophorus** sp. 1 (ANIC) only nested in areas of low ryegrass density (Figure 2). With one exception, all species were distributed independently of each other. Only **Pheidole** sp. J.D.M. 155 and **Pheidole** sp. J.D.M. 37 were significantly aggregated with respect to each other.

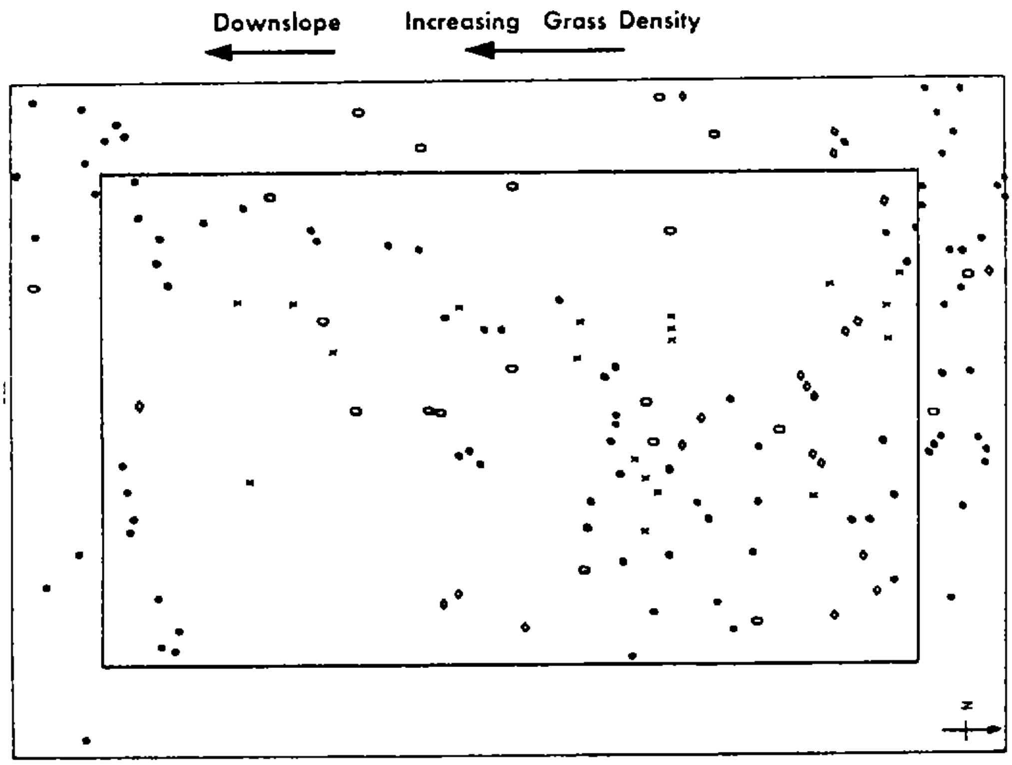

FIGURE 2

DISTRIBUTION OF ANTS IN THE 45m x 30 m QUARTERMAINE PASTURE PLOT. THE INNER RECTANGLE CIRCUMSCRIBES THE NESTS USED FOR THE NEAREST NEIGHBOUR ANALYSIS. KEY: ◇ , **MELOPHORUS** sp. 1 (ANIC); ●, **PHEIDOLE** sp. J.D.M. 155; x, **PHEIDOLE** sp. J.D.M. 37; **O, RHYTIDOPONERA INORNATA.**

Species		No. of nests	R^+	Spacing pattern
(a) Intraspecific				
Melophorus sp. 1 (ANIC)		17	0.3294*	aggregated
Pheidole sp. J.D.M. 37		19	0.3631*	aggregated
Pheidole sp. J.D.M. 155		55	0.3680*	aggregated
Rhytidoponera inornata		13	0.4999*	aggregated
(b) Interspecific				
Rhytidoponera inornata and;	*Melophorus* sp. 1 (ANIC)		1.2337	random
	Pheidole sp. J.D.M. 37		0.7267	random
	Pheidole sp. J.D.M. 155		0.7988	random
Melophorus sp. 1 and;	*Pheidole* sp. J.D.M. 37		0.9661	random
	Pheidole sp. J.D.M. 155		0.9970	random
Pheidole sp. J.D.M. 37 and;	*Pheidole* sp. J.D.M. 155		0.7535*	aggregated

TABLE 1

INTRASPECIFIC AND INTERSPECIFIC PATTERN ANALYSIS OF ANT SPECIES SHOWING THE RATIO OF OBSERVED TO EXPECTED MEAN DISTANCES BETWEEN COLONIES (R). SIGNIFICANCE VALUES (*) REPRESENT THE p <0.05 LEVEL.

Seed Removal by Ants

Wind removal of ryegrass seed from the seed depots was a problem during the December 1980 Butterworth plot seed taking trial. Consequently, these results were discarded and the plot was relocated at the more sheltered Quartermaine farm site (the site used for the 1979/80 pitfall trapping work). Sand paper was also attached to the seed depots to help overcome the problem of wind removal of seed. With the exception of gall shed, all results presented in this paper are from the Quartermaine site.

The raw data for the 1981 January and February seed removal trials are presented in Figure 3.

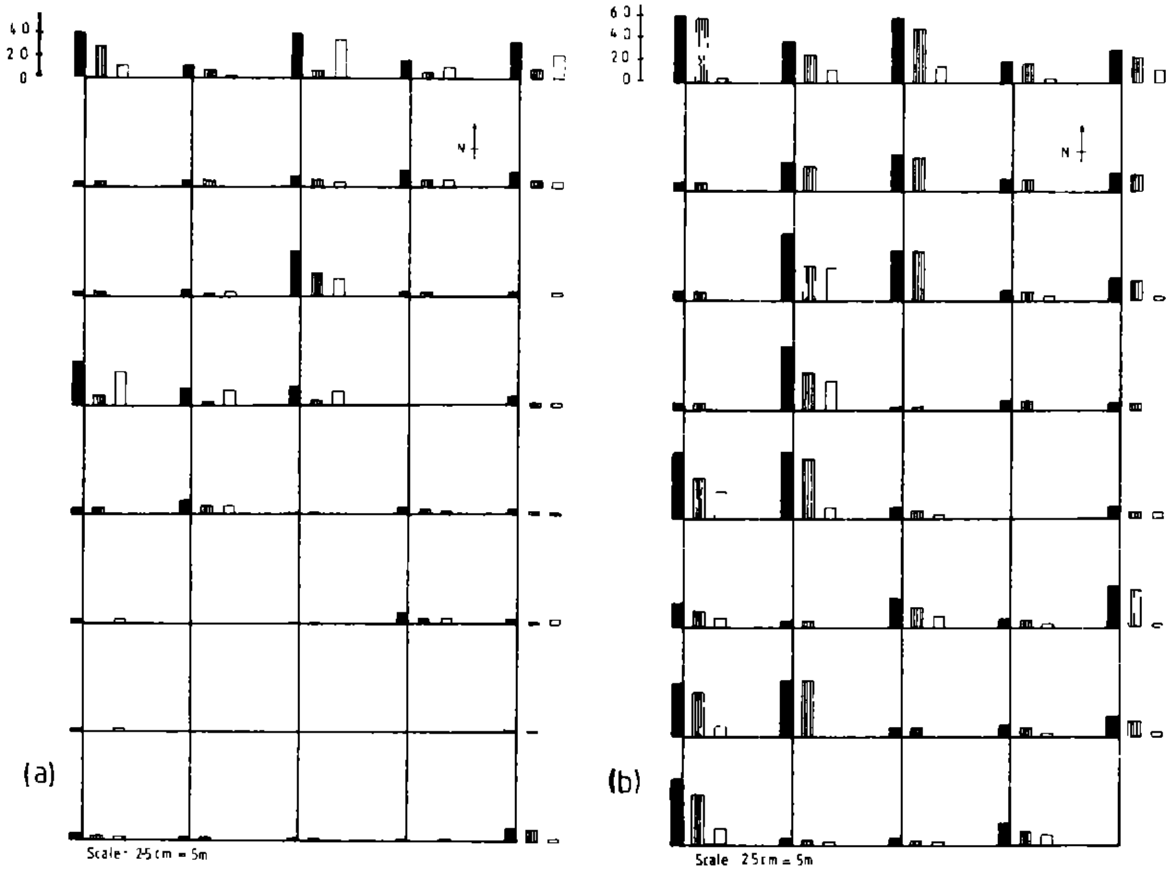

FIGURE 3

SEED REMOVAL PER DEPOT DURING, (a) JANUARY AND (b) FEBRUARY: THE LARGE RECTANGLE REPRESENTS THE INNER PLOT SHOWN IN FIGURE 2. EACH CORNER OF A SQUARE REPRESENTS A SEED DEPOT AND THE HISTOGRAMS SHOW THE RESULTS FOR THAT DEPOT.

KEY: □ SEED REMOVED AT NIGHT (8.00 AM TO 4.00 AM INCLUSIVE),
▤ SEED REMOVED DURING DAYLIGHT,
■ TOTAL SEED.

NOTE: FEBRUARY DATA INCLUDES SEED AND GALLS.

Twenty-two percent and 30% of the seed placed at depots were removed in 24 hours during the January and February experiments, respectively (Figure 4). Most seed was removed at night. However, as the morning and early evening soil temperatures were slightly lower in February (Figure 5), ant foraging was extended into these periods. During the February experiment seed removal per 2 hour intervals was negatively correlated with soil and ambient temperature ($p<0.05$ using Pearson correlation coefficient - Table 2). During both January and February seed taking was inversely related to rising temperature (Figure 6).

			New Seed Taken	
		Ambient Temperature (°C)	Relative Humidity (%)	Soil Temperature (°C)
Experiment No. 1	17/Dec/1980	0.613*	-0.499	0.671*
Experiment No. 2	15/Jan/1981	-0.516	0.425	-0.373
Experiment No. 3	14/Feb/1981	-0.597*	0.572	-0.641*

*Significant at $P<0.05$

TABLE 2

CORRELATION OF SEED TAKING WITH ENVIRONMENTAL PARAMETERS USING PEARSON'S CORRELATION COEFFICIENT.

Pheidole sp. J.D.M. 155 was most frequently observed taking seed (ninety-six observations), followed by **Pheidole** sp. J.D.M. 37 (twenty-six observations), **R. inornata** (ten observations) and **Melophorus** sp. 1 (ANIC)(eight observations). Other species which were occasionally observed taking seed included **Cardiocondyla nuda**, **Chelaner** sp. indet. and **Rhytidoponera violacea** (Table 3). Figure 7 gives the times when the four major species were observed taking seed. **Melophorus** sp. 1 (ANIC) only harvested during daylight while **R. inornata** was active over the full 24 hours. Both **Pheidole** spp. harvested at night although they were also active during early morning in February.

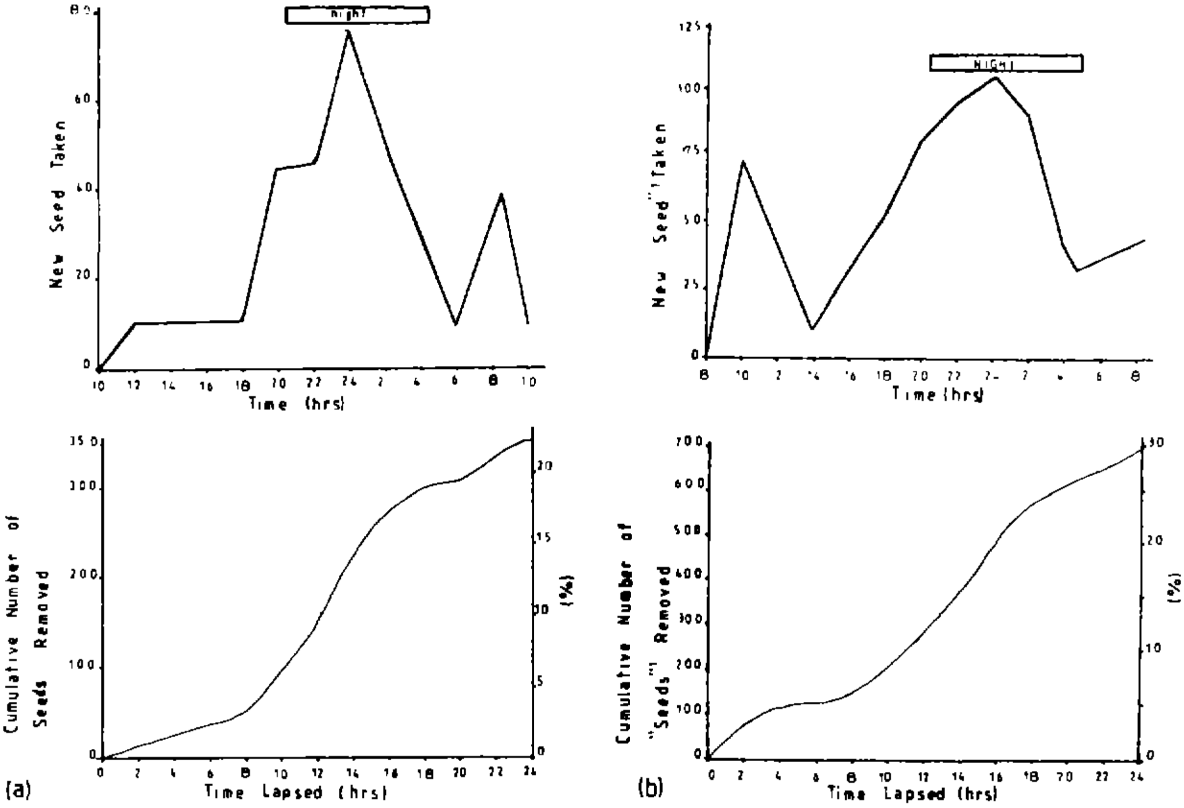

FIGURE 4

NEW SEED TAKEN AND THE CUMULATIVE NUMBER OF SEED REMOVED FOR
(a) JANUARY
(b) FEBRUARY.

"SEEDS"[1] INCLUDES SEEDS AND GALLS.

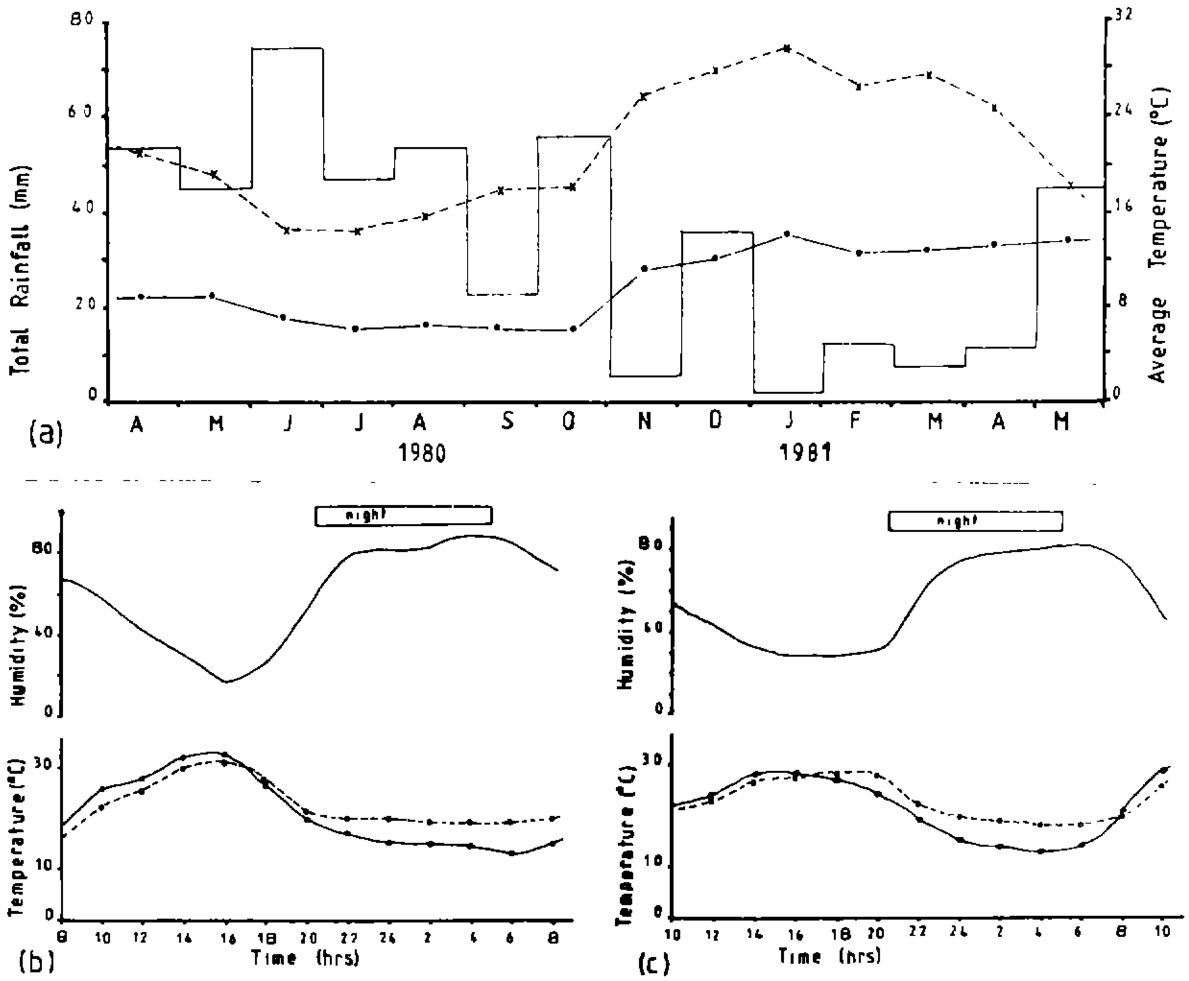

FIGURE 5

METEOROLOGICAL DATA DURING THE 1981 SEED TAKING EXPERIMENTS;
(a) MONTHLY VALUES, KEY: x---x, MAXIMUM AMBIENT TEMPERATURE; ●___●, MINIMUM AMBIENT TEMPERATURE; HISTOGRAM IS RAINFALL.
(b) READINGS FOR 2 HOUR INTERVALS DURING JANUARY;
(c) READINGS FOR 2 HOURLY INTERVALS DURING FEBRUARY. KEY: ●___● AMBIENT TEMPERATURE, o---o SOIL TEMPERATURE

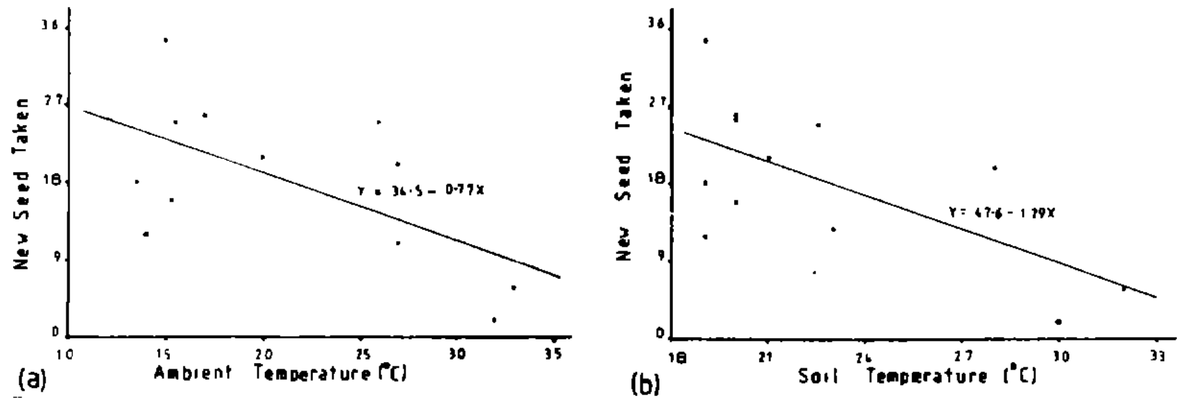

FIGURE 6

THE REGRESSION LINES OF NEW SEED TAKEN IN FEBRUARY WITH, (a) AMBIENT TEMPERATURE, (b) SOIL TEMPERATURE.

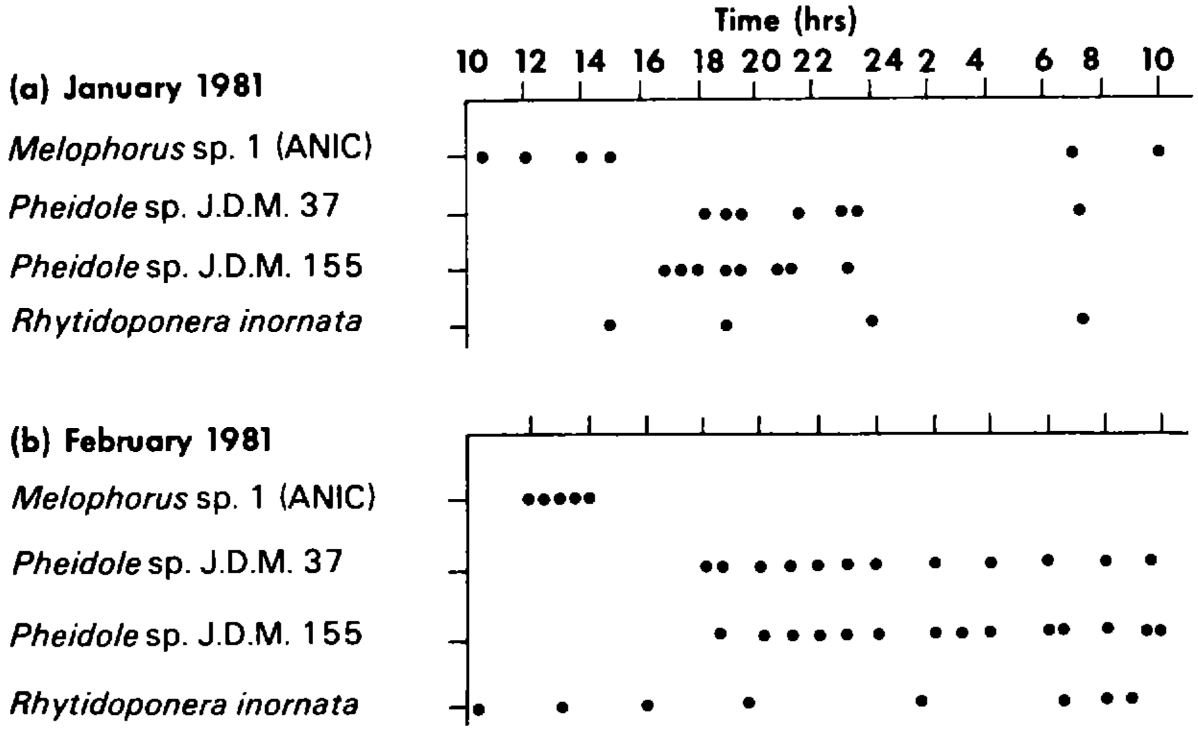

FIGURE 7

TIMES WHEN THE FOUR MAJOR SEED HARVESTING ANTS WERE OBSERVED TAKING SEED DURING THE JANUARY AND FEBRUARY 1981 EXPERIMENTS.

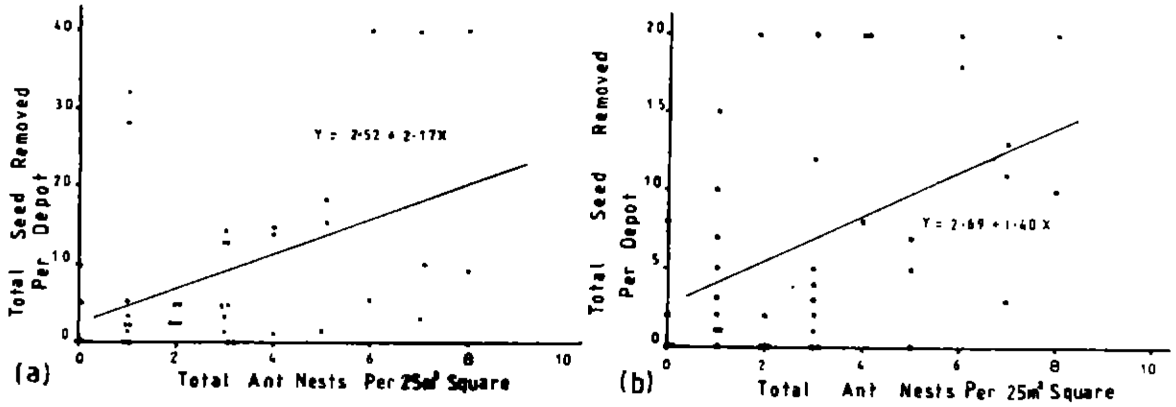

FIGURE 8

THE REGRESSION LINES OF SEED REMOVED PER DEPOT WITH TOTAL ANT NESTS PER 5 x 5 M SQUARE SURROUNDING EACH DEPOT FOR, (a) JANUARY (b) FEBRUARY.

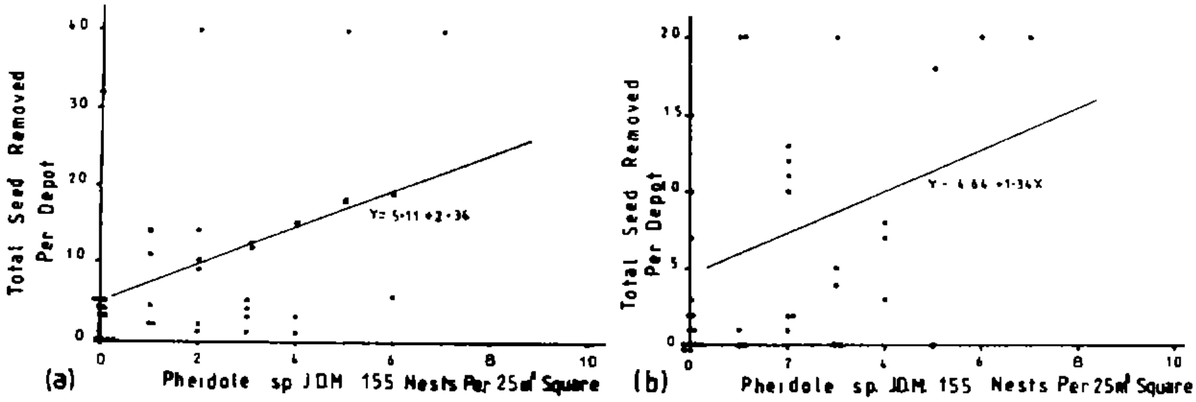

FIGURE 9

THE REGRESSION LINES FOR SEED REMOVED PER DEPOT WITH **PHEIDOLE** SP. J.D.M. 155 NESTS PER 5 x 5 M SQUARE SURROUNDING EACH DEPOT FOR, (a) JANUARY (b) FEBRUARY.

	Ant Nos.							
Species	Exp. No. 2 15/Jan			Exp. No. 3 14/Feb			Experimental Total	
	Major	Minor	Total	Major	Minor	Total	Actual	%
Cardiocondyla nuda						2	2	1.4
Chelaner sp. Kat. No. 7			3				3	2.1
Melophorus sp. 1	1	1	2	3	3	6	8	5.5
Pheidole sp. J.D.M. 37	1	6	7	1	18	19	26	17.8
Pheidole sp. J.D.M. 155	2	10	12	8	76	84	96	65.8
Rhytidoponera violacea						1	1	0.6
Rhytidoponera inornata			1			9	10	6.8
Total ants							146	

TABLE 3

ANT SPECIES OBSERVED TAKING SEED DURING THE JANUARY AND FEBRUARY 1981 EXPERIMENTS.

In order to assess the influence of each species on seed removal, the total seeds removed during the 24 hour period, the seeds removed at night and the seeds removed during the daytime were separately correlated with the numbers of ant nests of the four major species occurring in a 5 m x 5 m square surrounding each depot. Table 4 shows these results. The significant correlations were all relatively low although, when combined with the information in Figure 7, these correlations enable some interesting trends to be emphasised. Daytime seed removal during January was positively correlated with **Melophorus** sp. 1 (ANIC) nest density. Nightime seed removal was positively correlated with **Pheidole** sp. J.D.M. 155 density in January, and with **Pheidole** sp. J.D.M. 37 density in February. **Pheidole** sp. J.D.M. 155 density was positively correlated with daytime seed removal during February which reflects the tendency for this species to forage during daylight in this month (Figure 7). In both experiments total seed removed was positively correlated with total ant nests in the depot surrounds (Figure 8).

The influence of the **Pheidole** species upon seed taking is further emphasised in Figures 9 and 10. From these figures, the regression lines of seed removal with the **Pheidole** species indicates that seed removal increased with increasing **Pheidole** nest density. However, **Pheidole** sp. J.D.M. 155 exerted the greatest influence upon seed removal as only 1.0 seeds per depot were removed during the periods when **Pheidole** sp. J.D.M. 155 was inactive (Table 5). The data in Table 5 were arranged to show that seed taking was greatly reduced when **Pheidole** sp. J.D.M. 155 was not removing seed. However, due to the large standard deviation of the number of seeds removed from each depot during each period analysed, these results do not always equate to the total amount of material ("seeds") removed during the 24 hour choice experiment.

Species	Experiment No. 2 15 Jan, 1981			Experiment No. 3 14 Feb, 1981		
	Day	Night	Total	Day	Night	Total
Melophorus sp. 1 (ANIC)	0.381*	0.279	0.326	-0.048	0.044	0.044
Pheidole sp. J.D.M. 37	0.025	-0.030	0.012	0.040	0.388*	0.247
Pheidole sp. J.D.M. 155	0.227	0.340*	0.330*	0.406*	0.038	0.388*
Rhytidoponera inornata	0.041	-0.004	-0.021	0.085	0.088	0.138
All species	0.301	0.371*	0.369*	0.347*	0.228	0.440*

TABLE 4

CORRELATION COEFFICIENTS FOR COMPARISONS BETWEEN TOTAL SEED, NIGHTIME SEED AND DAYTIME SEED TAKEN AND THE NUMBER OF NESTS OF PARTICULAR SPECIES IN THE 5 x 5 M SQUARE SURROUNDING EACH DEPOT. SPEARMAN`S RANK CORRELATION WAS USED AND SIGNIFICANCE VALUES (*) REPRESENT THE $p < 0.05$ LEVEL.

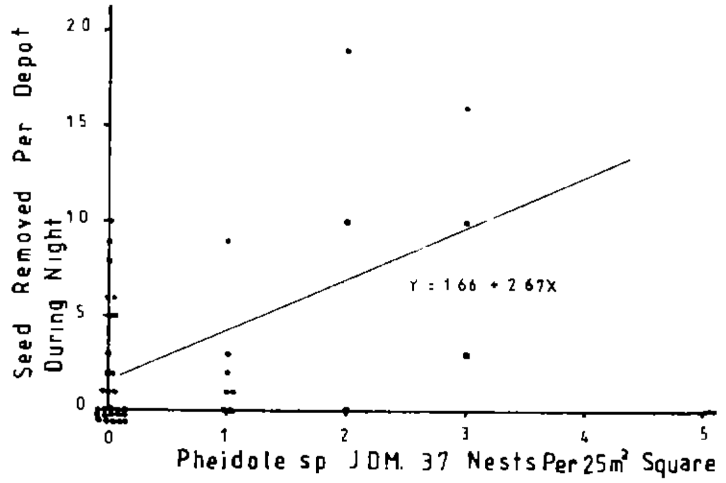

FIGURE 10

THE REGRESSION LINE FOR SEED REMOVED PER DEPOT DURING NIGHT AGAINST THE NUMBER OF **PHEIDOLE** SP. J.D.M. 37 NESTS PER 5 x 5 M SQUARE SURROUNDING EACH DEPOT. THE DATA IS FOR FEBRUARY.

		Material taken by ants per depot in 24hrs.		
		Seed	Bacterial Galls	Nematode Galls
Total Material	$\bar{X}$	5.80	6.25	6.75
	SD	7.01	6.97	6.61
Material removed during day (4.00am to 8.00pm)	$\bar{X}$	4.15	4.10	4.60
	SD	5.95	5.22	5.73
Material removed during night (8.00pm to 4.00am)	$\bar{X}$	3.00	3.10	3.35
	SD	4.62	4.52	4.79
Active period of <u>Pheidole</u> sp. J.D.M. 155 (6.00pm to 10.00am)	$\bar{X}$	5.48	6.45	6.60
	SD	7.01	6.38	6.29
Inactive period of <u>Pheidole</u> sp. J.D.M. 155 (10.00am to 6.00pm)	$\bar{X}$	1.00	1.20	1.10
	SD	1.57	1.90	2.97

TABLE 5

THE EFFECT OF **PHEIDOLE** sp. J.D.M. 155 UPON "SEED" REMOVAL DURING THE FEBRUARY CHOICE EXPERIMENT.

Table 6 shows the raw data for the nest analyses. These data are summarised in Table 7 giving some insight into the fate of harvested seed. The mean seed (galls included) in nest and midden indicate that the bulk of seed is placed around the midden of the nest. Whether these deposits represent rejecta or stored material is as yet unknown. Considerable amounts of husk were obtained from the nests and middens of all species. The amount of midden material (which contained both husks and seeds) was subjectively scaled from zero, when the midden was absent, to ten, for a nest with a very large midden mass. The subjective ten point scale shows that the two **Pheidole** spp. were associated with the largest amount of husk (Table 7). This indicates that considerable amounts of seed had been consumed prior to sampling. It should be noted that samples were taken from a grazed area where sheep were "competing" with ants for seed; Stynes (unpublished data) has collected 6,973 ± 1,704 seeds or galls (n = 4) from, what were probably, **Pheidole** sp. J.D.M. 155 or **Chelaner** sp. indet. nests situated in ungrazed pasture.

Seed Versus Gall Preference by Ants

The removal rates for healthy seed and nematode and bacterial galls were not significantly different during the February choice experiment (Figure 11). This result was substantiated by analysis of the composition of seed and galls in nests of the four major harvesting ants (Table 8). The relative composition of these items in bare ground was 56.1% healthy seed, 30.5% bacterial galls and 13.4% nematode galls. The percentage compositon of items in ant nests did not differ from that expected (using chi-square analysis) on the assumption that composition of seed in nests reflects that on bare ground (Table 8).

Melophorus sp. 1

Nest No.	All material present in Midden	 Nest	 Total
1	73	48	121
2	15	5	20
3	176	8	184
4	4	14	18
5	32	13	45
$\bar{X}$	60	17	77
SD	69	21	72

Pheidole sp. J.D.M. 37

Nest No.	All material present in Midden	 Nest	 Total
1	51	24	75
2	130	79	209
3	10	4	14
4	855	179	1,034
5	70	32	102
$\bar{X}$	223	63	286
SD	355	70	423

Pheidole sp. J.D M. 155

Nest No.	All material present in Midden	 Nest	 Total
1	2	9	11
2	13	11	24
3	51	3	54
4	15	31	46
5	35	8	43
$\bar{X}$	23	12	35
SD	19	10	17

Rhytidoponera inornata

Nest No.	All material present in Midden	 Nest	 Total
1	70	11	81
2	33	3	36
3	43	26	69
4	176	16	192
5	85	27	112
$\bar{X}$	81	16	98
SD	56	10	59

TABLE 6

NEST ANALYSES FOR THE SEED TAKING ANTS, SEED AND GALLS COMBINED.

Species	Mean 'seed' content Midden	 Nest	 Total	Percentage composition of 'seed' Healthy seed	 Bacterial gall	 Nematode gall	Midden mass
Bare ground	?	?	16	56.1	30.5	13.4	-
Melophorus sp. 1 (ANIC)	60	17	77	58.8	27.6	13.6	1
Pheidole sp. J.D.M. 37	223	63	286	71.0	21.9	7.1	7
Pheidole sp. J.D.M. 155	23	12	35	47.5	36.9	15.6	9
Rhytidoponera inornata	81	16	98	50.2	35.6	14.2	3

TABLE 7

MEAN NUMBER OF SEEDS RETRIEVED FROM NESTS AND MIDDENS OF THE FOUR MAJOR HARVESTER ANTS. THE MIDDEN MASS IS THE AMOUNT OF HUSK SUBJECTIVELY SCALED FROM 0 (ABSENT) TO 10 (VERY HIGH). THE PERCENTAGE COMPOSITION OF HEALTHY SEED, BACTERIAL GALLS AND NEMATODE GALLS IS ALSO SHOWN.

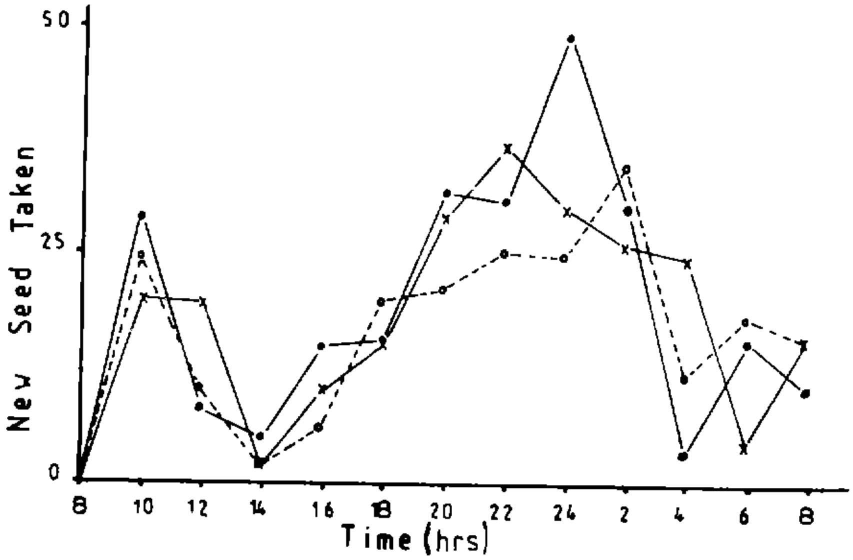

FIGURE 11

NEW SEED TAKEN DURING THE FEBRUARY CHOICE EXPERIMENT FOR DETERMINATION OF ANY SEED/GALL PREFERENCES DISPLAYED BY SEED TAKING ANTS. o---o SEED, x___x BACTERIAL GALLS, ●___● NEMATODE GALLS.

Ant Species	Seed	Galls	$^{*}x^2$value
Control	9.2	7.2	—
Melophorus sp. 1:			
observed	42.6	35.0	0.09
expected	42.8	34.8	
Pheidole sp. J.D.M. 37:			
observed	211.4	75.4	2.14
expected	208.7	78.1	
Pheidole sp. J.D.M. 155:			
observed	16.0	19.6	0.54
expected	17.3	18.3	
Rhytidoponera inornata:			
observed	49.6	48.4	0.24
expected	50.04	47.6	

$^{*}x^2_{0.05(1d.f.)} = 3.84$

TABLE 8

ANALYSES OF THE NESTS OF SEED TAKING SPECIES, FOR DETERMINATION OF ANY SEED/GALL PREFERENCES, MIDDEN AND NEST COMBINED.

DISCUSSION

Comparison of the Katanning results with other data on Western Australian pasture seed removal by ants was not possible as this paper is the first published account of seed theft from Western Australian pastures.

The present findings indicate the existence of a populous and relatively species-rich ant community in ryegrass pastures. At least four of the species are significant seed harvesters although observations in adjacent pastures indicate that **Chelaner** sp. indet. may also remove significant quantities of seed. Taking all observations into account the ranking of seed harvester importance runs from **Pheidole** sp. J.D.M. 155, **Pheidole** sp. J.D.M. 37, **Melophorus** sp. 1 (ANIC) to **R. inornata**, the least important species. The foraging activity of most species is closely synchronous with seed/gall drop (Figure 1) and this probably reflects the dependence on seed in the diet of most pasture ants (Majer, in press). Small quantities of arthropod fragments were retrieved from nests of the four major harvesters indicating some degree of omnivory.

Despite their aggregated intra-specific nest patterns, nests were distributed over a broad range of the pasture. **Melophorus** sp. 1 (ANIC) nest density was lower under areas of high grass density. The lack of inter-specific overdispersion probably reflects the presence of plentiful food resources and also the low age of the ant community.

Many workers (Campbell and Gilmour, 1979; McGowan, 1969) have indicated the importance of seed harvesting by ants in the removal of surface sown/distributed seed. Results from pasture seed depots at Katanning suggested that between 20-30% of the ryegrass seed was removed in 24 hrs (Figures 6 and 7). These figures may well be inflated due to the seed being placed in concentrated clumps (depots). Many harvesting ants display well developed foraging behaviour. It could be argued therefore, that seed removal rates may in part be due to the harvesting ants "learning" that seed was available in a certain area and thus, increasing foraging in that area (Johns and Greenup, 1976b). A further factor which may be of importance when considering the Katanning seed theft rates, was the heavy winter grazing by livestock at the Quartermaine site. Heavy livestock grazing is one of the control methods adopted to reduce the number of ryegrass plants in the paddocks. As a result, the amount of seed set is reduced and this may influence the availability of food to the ants. Seed taking rates may increase when the ants are supplied with their preferred food, ryegrass seed. Interpretation of the depot seed theft experiments should therefore be approached with the realisation that it is not a natural situation. Nevertheless, the level of ryegrass seed removed over 24 hours is of similar magnitude to that recorded in pastures by other Australian workers (61% after 3 days [Russel et al., 1967], 35% after 24 hours [Johns and Greenup, 1976a], and 60-100% after 14 hours [Mott and McKeon, 1977]). Though the

Katanning figures may well be inflated due to seeds being placed in concentrated patches they, and Stynes` (unpublished) analyses of nests in ungrazed areas, suggest that ants are significant agents in the fate of ryegrass seed and galls in Western Australian pastures.

As with other studies (Johns and Greenup, 1976a, 1976b), this investigation indicated that seed taking by certain species was closely correlated with soil and ambient temperature. Seed taking by both **Pheidole** species was inversely related to soil and ambient temperature (Table 2 and Figure 6). On cooler days, the **Pheidole** ants` foraging activity was extended for longer periods in the mornings and evenings, thus increasing harvesting time. **Melophorus** sp. 1 activity increased with increasing temperature and its` foraging activity was limited to the hottest part of the day (Table 2). The effect of temperature upon seed removal was further emphasised by Mott and McKeon (1977) who artificially shaded **Pheidole** nest openings which stimulated earlier foraging. Johns and Greenup (1976a) suggest that the strong positive correlations between soil temperature (up to 25oC) and seed theft rate could be due to either or both of the following factors: 1) theft rates being partly dependent on recent air temperatures such that thermal inertia causes soil temperatures to be a function of both current and previous air temperatures, or 2) foraging activity may be sensitive to gallery temperatures. Johns and Greenup (1976a) also substantiated that seed taking is not influenced by relative humidity. This reflects the findings of this investigation.

An interesting contrast was indicated between the final destination of harvested seed as described by Johns and Greenup (1976a) and in this study. Johns and Greenup found that ants of the genus **Pheidole** stored seed in underground galleries. In the Katanning experiments, seed taken by **Pheidole** sp. J.D.M. 155 and **Pheidole** sp. J.D.M. 37 was stored in the above ground midden (Tables 6 and 7). Campbell (1966) also suggests that seed is not stored above the ground in **Pheidole** nests. The storing of seed above ground may be important in the ecology of annual ryegrass toxicity, as some seeds and galls remain placed in an ideal position for later `germination`.

Pheidole sp. J.D.M. 155 (93 nests), the most abundant of the four seed taking species at the Quartermaine site, had the greatest influence on seed taking as it removed vast quantities of seed. Analaysis of whole seed present in **Pheidole** sp. J.D.M. 155 nests (Table 7) indicated that little of this seed escaped being eaten by the ants. Therefore **Pheidole** sp. J.D.M. 155 has the potential to remove large numbers of toxic and non-toxic seed from the paddocks, and to consume the seed as food. Berg (1975) and Majer (in press) have reported that **Pheidole** species mainly feed on entire seeds. This would account for the high amount of husk material, and low numbers of whole seed found in the **Pheidole** sp. J.D.M. 155 nest middens.

An important factor influencing seed harvesting from grasslands, is the selection of seed species by ants. McGowan (1969) suggests that species of **Pheidole, Chalcoponera,** and **Iridomyrmex** all harvest ryegrass seed in preference to the other annual grasses present. Often, comparatively little seed of barley grass (**Hordeum leporinum**) or subterranean clover (**Trifolium subterraneum**) may be removed. It appears that both seed size and seed weight influence the selection of seeds by ants (McGowan, 1969). The relatively small, light ryegrass seeds are preferentially removed by ants instead of the heavier seeds, such as subterranean clover. However, it is important to realise that the ants vary their diets according to the availability of food. Due to the heavy control by livestock grazing at the Quartermaine site, the ryegrass content of the paddocks and therefore the ant nests was low. As barley grass and great broome (**Bromus diandrus**) were the dominant components of pastures at this site, and since livestock do not readily eat the dry plants, the nest middens contained large amounts of barley grass and great broome seeds. Most of the ant nests examined from the Quartermaine site contained seeds of barley grass, great broome, capeweed (**Arctotheca calendula**), subterranean clover, and other annual grasses, in addition to those of ryegrass. Seeds other than ryegrass were not included in the nest analyses as this investigation was mainly interested in annual ryegrass toxicity.

The likelihood of livestock being affected by annual ryegrass toxicity depends upon three factors: 1) the density of ryegrass in the pasture which determines the number of infection sites, 2) the density of the causal organism, and 3) the quantity of toxic material consumed (Stynes and Wise, 1980). Any event which reduces one or all of these factors may be important in controlling annual ryegrass toxicity. It is in this area that the role of seed harvesting ants may be critical as they may limit the amount of ryegrass material present in the paddocks.

The selection of seed species and the seed/gall selection displayed by the seed harvesting ants at Katanning is of importance. Ants may selectively remove ryegrass seed from the paddocks and thus, the amount of toxic ryegrass material would also be reduced. However, as the ants displayed no preferences between the lighter bacterial and nematode galls and healthy seed (Table 8 and Figure 11) the infected galls would be removed at a similar rate to that of the ryegrass seed. Of equal importance, is the reduction of available ryegrass seed for germination the following year. The high degree of ryegrass seed consumption, and the unselective inclusion of galls in material removed, means that ants would tend to reduce the toxic material available for consumption by stock or infecting the next crop of ryegrass.

The influence of ants in the dispersal of ryegrass toxicity is as yet unknown. By concentrating galls the distribution of toxicity in next year`s pasture may be patchy; the implications of this are not yet known. Another possible effect may arise from stock consuming infected material aggregated on the nest middens.

Stynes and Wise (1980) indicate that between 76% and 95% of livestock deaths caused by annual ryegrass toxicity, occur on fields grazed within 12 months of being cultivated and sown. The risk of losses falls considerably in the second year and is negligible after 3 or more years of continuous pasture. Stynes and Wise (1980) found that cultivation and cropping had no affect on gall production. The Katanning results suggest that infrequent, shallow cultivation has little affect upon ant density. As a result, the influence of cultivation on ant nests is probably not an important factor in the outbreak of ryegrass toxicity.

Under cereal cropping annual ryegrass density in the crop increases while most other grasses disappear. Thus, when the land is returned to pasture, ryegrass is often the dominant component. Other grasses may reappear after one or two seasons and gradually replace the ryegrass with time. As seed taking is strongly correlated to soil temperatures, the shading effect of the dense cereal stubble may be important in determining the length of the foraging periods of the ants. Seed harvesting ants have a minimum temperature which must be reached before foraging commences (Mott and McKeon 1977). Further investigation may indicate that the cereal stubble increases the time needed to attain this temperature. As a result of reduced foraging activity, the amount of ryegrass material removed by ants may be reduced. The cereal stubble may also physically hinder ant activity (McGown, 1969), and so may influence the disease epidemiology.

One further management practice which should be considered is the effects of fire on gall production (Stynes and Wise, 1980). Burning of cereal stubble in the autumn, although having no influence on total gall production, did significantly reduce the number of galls that became colonised by bacteria (the toxic agent). Autumn burning reduced the risk of toxicity in subsequent pastures by reducing the bacterial galls by up to 85% (Stynes and Wise, 1980). The same effect is achieved by autumn burning of pastures, provided sufficient heat is generated by the fire.

The effects of such fires upon seed harvesting ants would be negligible, for the following reasons: 1) ant activity during the autumn is considerably reduced (Figure 1), so most ant activity would be confined to the below ground nest and not on the surface, and 2) heat penetration through the soil would be low and little or no effect would be felt in the nest galleries.

The effects of fire and dense cereal stubble upon seed harvesting ants merits further investigation. Burning of pastures before cropping and the removal of the dense stubble after harvest by fire, would reduce the number of bacterial galls formed in the subsequent pastures following cropping. As fire appears to have little effect upon the ants, the introduction of burning before and after cropping as an agricultural practice, may well be worthwhile.

The Katanning results indicate that in the pastures of south-west Western Australia, seed harvesting ants have the capacity to remove large quantities of seed. Both **Pheidole** species, in particular **Pheidole** sp. J.D.M. 155, exert the greatest influence upon seed removal rates in the study area. Unlike other areas of Australia, such as Victoria and New South Wales, where seed removal by ants is often detrimental to pasture establishment, seed removal by ants in the Katanning area may be beneficial. This is because seed taking from this area may play a critical role in controlling the outbreak and decline of the livestock disease annual ryegrass toxicity.

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