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POLLINATORS OF THE JERVIS BAY LEEK ORCHID, *Prasophyllum affine*, AT VINCENTIA, NSW – NECTAR RESOURCES

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SUMMARY

This section summarises the findings of the study in relation to the Project Objectives.

Objective 1. Determine the range of nectar sources utilised by *Ariphron* sp. and Pepsinae.

- Ariphron *sp. and Pepsinae* were found to be members of a large guild of generalised anthophilous (flower loving) insects that exploit the flowers of Tea Trees, Eucalypts and Hakea at Vincentia for nectar.

- The dominant species utilised were Yellow Tea Tree (*Leptospermum polygalifolium*) and Dagger Hakea (*Hakea teretifolia*). Less important hosts were Port Jackson Mallee (*Eucalyptus obstans*) and a Tea Tree (*Leptospermum continentale*). Potential, but unverified hosts include Tickbush (*Kunzea ambigua*) and *Leptospermum juniperinum*.

Objective 2. Map the distribution of these resources adjacent to *Ariphron* sp. breeding areas.

- Yellow Tea Tree and Dagger Hakea were widespread and abundant across the entire study area, and carried heavy loads of blossom with abundant nectar.

- Flowering times varied across the site giving a spread of nectar availability from each of the widespread species as different patches came into bloom successively. Flowering started in the sedgeland areas, then moved outwards into the adjoining heaths and forests, extending flowering over about two months for Yellow Tea Tree and Dagger Hakea.

- Tickbush distribution was limited to the south western corner of the study area and had a short flowering period of about one month. Port Jackson Mallee was very patchily distributed with different patches having discrete flowering periods. Although widespread, plants of *Leptospermum continentale* were distributed at low density, were generally small and carried relatively few flowers.

Objective 3. Determine the feeding flight distances of *Ariphron* sp*.*

- Mark and recapture experiments with male *Ariphron* sp. were unsuccessful due to lower abundance of this species in 2004. Only 68 males were marked, versus 187 in 2003, and only one was recaptured.

Objective 4. If possible, rank the importance of each nectar source species and feeding area for *Ariphron* sp. colonies that contribute to pollination of JBLO.

- The flight period of *Ariphron* sp. spanned the latter part of Yellow Tea Tree flowering and finished in the middle of Dagger Hakea bloom.

- A period of low nectar availability of 10 to 12 days was identified between the end of Yellow Tea Tree flowering and the beginning of Dagger Hakea. Nectar was available in more limited quantities in this period from Port Jackson Mallee, Tickbush and *Leptospermum continentale*, which were much less abundant and patchily distributed.

- Nectar feeding by *Ariphron* sp. was not randomly distributed across the available nectar sources. Rather, feeding observations were highly clustered in three patches, all of which were located within 40 to 60 m of known *Ariphron* sp. breeding areas. Two of these clusters were associated with the breeding area closest to the main *Prasophyllum affine* colony adjacent to the Leisure Centre drive. Both clusters are contained within the Environment Protection Zone of the proposed development.

Objective 5. Determine the relative importance of minor disturbance areas and undisturbed groundcover for the breeding of *Ariphron* sp.

- Experiments to compare patrolling activity by male *Ariphron* sp. in disturbed and undisturbed habitats were prevented by overcast weather conditions on all days but one. The data obtained on this day was consistent with previous observations that male patrolling is most prevalent in disturbed areas. However, the data was insufficient to quantify the degree of breeding in undisturbed habitats.
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Objective 6. Determine the ability of *Ariphron* sp. to fly under grids mimicking the proposed gridded roadway.

- Patrolling behaviour of male *Ariphron* sp. was not disrupted by one metre wide garden lattice, giving 60 percent light reduction, suspended 1.5 m above the flyway. However, flight was inhibited for 30 percent of individuals when the lattice was lowered to heights of 0.6 and 0.2 m. While these results do not preclude flight by *Ariphron* sp. below a suspended and gridded roadway, the effect of a much longer shadow width than one metre remains unknown.

Objective 7. Provide recommendations on critical feeding and breeding habitat for the pollinators of JBLO and its management to ensure the ongoing survival of the Vincentia JBLO populations.

RECOMMENDATION

1. That rehabilitation and ornamental plantings on the Vincentia development site include nectar source species suited to generalist nectar feeding insects. In particular, species that flower prolifically in the gap between Yellow Tea Tree and Dagger Hakea should be well represented, i.e. Port Jackson Mallee, *Eucalyptus obstans* and Tickbush, *Kunzea ambigua*.

CONCLUSIONS

1. The redesigned Vincentia development as per the masterplan of December 2004 provides adequate protection for the Jervis Bay Leek Orchid, its pollinators and the resources required to maintain pollinator populations, particularly nectar resources for adults and areas for breeding.

2. It is not possible to conclude on the available information that an elevated and gridded roadway 1.5 m high will allow movement of *Ariphron* sp. from one side to the other.

TECHNICAL SUMMARY

This section summarises the main scientific findings of the study.

- The study aimed to determine the range of nectar resources utilised by insects, particularly the thynnine wasp, *Ariphron* sp., known to pollinate the Jervis Bay Leek Orchid, *Prasophyllum affine*, their distribution in space and time, and the relative importance of each nectar providing species. Additional aims were to obtain more information on distances moved by *Ariphron* sp. males to nectar sources, the importance of undisturbed habitat for breeding and the effects of overhead shading on movement.

- Pollinators of *P. affine* were found to utilise two main nectar sources, Yellow Tea Tree, *Leptospermum polygalifolium* and Dagger Hakea, *Hakea teretifolia*. Less important were Port Jackson Mallee, *Eucalyptus obstans* and *Leptospermum continentale*.

- The pollinators of *P. affine* were part of a large complex, comprising many species of Hymenoptera, Diptera and Coleoptera, that visited flowers of the above four species as well as Tickbush, *Kunzea ambigua* and *Leptospermum juniperinum*. It is considered the last two species are also potential food sources for pollinators of *P. affine*.

- Observations showed there is a continuous nectar supply across the whole site from five myrtaceous species and Dagger Hakea between about mid September and mid to late January, which spans the adult phases of the *P. affine* pollinator life cycles. However, not all six species are widespread on the study area and parts of the site may undergo periods of nectar scarcity. The most widespread species are Yellow Tea Tree and Dagger Hakea, with the main period of nectar scarcity being about 10 to 12 days in mid November, between the end of Yellow Tea Tree flowering and the commencement of Dagger Hakea.

- Observations of nectarivory by *Ariphron* sp. were concentrated in three discrete clusters within the study area, all within a minimum of 40 to 80 m of known *Ariphron* sp. breeding areas.

- Unfavourable overcast weather conditions prevented sufficient observations being made to draw conclusions on the relative importance of undisturbed versus lightly disturbed habitat for breeding of *Ariphron* sp. However, the results confirmed the prevalence of patrolling males in areas of minor disturbance.

- Partial shading by one metre wide overhead grids was found to have no effect on the patrolling behaviour of *Ariphron* sp. males if the grid was 1.5 m above the ground. However, inhibition of movement below the grid occurred if it was lowered to 0.6 or 0.2 m.

- The main period of flowering by *Prasophyllum affine* at the main Vincentia colony commenced at the beginning of November 2004 and peaked by 14 November. Flowering was a little earlier in 2004 than in 2003 and later than in 2001.

- Pollinators removed pollinaria from over 30 percent of open *P. affine* flowers at the peak of anthesis in mid November when eight percent of flowers had also been pollinated. Overall, 52.5 percent of flowers went on to develop seed pods, though 44.8 percent were medium or small indicating low levels of pollen deposition. Only 7.7 percent developed large pods indicating high pollen loads on the stigma. The levels of pollinaria removal and pollination at peak flowering were lower in 2004 than in 2003 and similar to 2001.

INTRODUCTION

The Jervis Bay Leek Orchid (JBLO), *Prasophyllum affine*, is listed as endangered under the New South Wales *Threatened Species Conservation Act 1995* and the Commonwealth *Environment Protection and Biodiversity Conservation Act 1999*. Its current known distribution is limited to a few locations around Jervis Bay, one of which is the proposed Stockland commercial and residential development site at Vincentia.

A Recovery Team for JBLO was established by the New South Wales National Parks and Wildlife Service, as provided under the TSC Act, and a draft Recovery Plan for *P. affine* was issued in April 2003. A major issue identified by the Recovery Team for conservation of the existing JBLO populations at Vincentia is to determine the size of the area needed for their long term viability. In particular the Recovery Team is keen to ensure that sufficient area is set aside to maintain viable populations of the JBLO pollinators, including all the resources needed for all stages of their life cycles. Accordingly, the developers, first Realty Realizations and later Stockland, have funded research to determine what insects pollinate JBLO, how they are distributed across the site and what other food plants they utilise.

Studies on JBLO were carried out during the flowering seasons in 2001 (Bower 2002) and 2003 (Bower 2004). The 2001 study determined that male Flower Wasps (Thynninae) were the dominant pollinators of JBLO, with Spider Wasps (Pepsinae) the only other important group. At Vincentia the most important pollinator by far was a small undescribed thynnine wasp in the genus *Ariphron*. The 2003 study confirmed the importance of *Ariphron* sp. as the dominant pollinator of JBLO at Vincentia and determined the distribution of its main breeding populations. Limited information on the distances travelled by pollinators to feed on JBLO was also obtained. The main source areas of pollinators for the Vincentia JBLO colonies were determined from this data, which was used in part to define the size and shape of the proposed environmental protection zone.

Following the 2003 study, the JBLO Recovery Team considered that additional information was needed on three areas of concern:

1. Whether the proposed environmental protection zone included sufficient nectar resources to maintain pollinator populations throughout their flight periods, i.e. before and after the flowering of JBLO.

2. There was also some concern that the importance of undisturbed habitat, versus minor disturbance areas, for the breeding of *Ariphron* sp. may have been underestimated due to the difficulty of detecting wasps in undisturbed vegetation.

3. Whether pollinators would fly under gridded elevated roadways between areas of habitat on either side, as proposed for a bridge over the creek on the eastern edge of the environmental protection zone.

Stockland agreed to fund a supplementary study in spring and summer 2004 with the following objectives.

OBJECTIVES

1. Determine the range of nectar sources utilised by *Ariphron* sp. and Pepsinae.

2. Map the distribution of these resources adjacent to *Ariphron* sp. breeding areas.

3. Determine the feeding flight distances of *Ariphron* sp.

4. If possible, rank the importance of each nectar source species and feeding area for *Ariphron* sp. colonies that contribute to pollination of JBLO.

5. Determine the relative importance of minor disturbance areas and undisturbed groundcover for the breeding of *Ariphron* sp.

6. Determine the ability of *Ariphron* sp. to fly under grids mimicking the proposed gridded roadway.

7. Provide recommendations on critical feeding and breeding habitat for the pollinators of JBLO and its management to ensure the ongoing survival of the Vincentia JBLO populations.

Detailed background information on the biology of Leek Orchids and the main groups of insects found to pollinate them is given in Bower (2002, 2004).

METHODS

Feeding observations.

Systematic observations were made of all species of flowering plants at Vincentia to determine which are utilised by *Ariphron* sp. and Pepsinae. This was done by conducting 30 min searches within defined areas and recording the numbers of all nectar-feeding insect species according to the plants being utilised. Insects were recorded to the lowest taxonomic level possible from field observation at a distance of 0.5 to 1 m from the subject. All insect visitors were recorded in order to determine to which of the nectar-feeding insect guilds present in the study area *Ariphron* sp. and Pepsinae belonged. This allowed potential nectar sources for *Ariphron* sp. and Pepsinae to be defined by guild as well as by direct observation, in case direct observations of *Ariphron* sp. or Pepsinae on some plants were infrequent. From these data the relative levels of visitation by *Ariphron* sp. and Pepsinae were ranked according to plant species, allowing the most important nectar sources to be determined. Observations of nectar feeding commenced each day as soon as temperatures were high enough for insect activity, which varied between 8 and 11 am on most days, and continued until temperatures fell too low in the afternoon, usually around 4 to 5 pm.

Since *Ariphron* may also feed on the honeydew secretions of psyllids and leafhoppers, attempts were made to locate psyllid and leafhopper colonies and monitor visitation by *Ariphron* sp. to them. Foliage and twigs of taller shrubs and trees was examined with a pair of binoculars (8×40) for the presence of honeydew secreting insects.

Feeding flight distances.

Ariphron sp. males were marked in order to determine how far they fly from their breeding areas to food sources. Methods of marking *Ariphron* sp. were similar to those successfully employed in 2003, viz. the use of small drops of coloured Tippex liquid paper on the insect's thorax. Since coloured Tippex or similar is no longer available, non-toxic Chromacryl® students acrylic paint was used instead. Males were captured using a sweep net as they patrolled for females in breeding colony areas. Males from each breeding colony were marked with a different colour. A 50 m tape measure was used to determine the minimum distances flown by each marked male recaptured or observed at each nectar source.

Breeding colony distribution.

Ten 100m transects were established perpendicular to minor tracks at the locations of known breeding colonies of *Ariphron* sp. The transects extended 50m on either side of each colony. Five minute observations for patrolling *Ariphron* sp. males were made at ten metre intervals along each transect, repeated at least once. Since the low flying (10 cm above the groundcover) males can be very difficult to detect against a background of dense vegetation, the observations were made early and late in the day when the sun is low in the sky and sunlight reflects from the insect's wings. When patrolling males were detected in new areas, samples were taken to verify their identity.

Unfortunately, except for one day, the weather conditions were unfavourable throughout the main study period in November with cloud blocking out the direct sunlight needed.

Evaluation of the effects of an overhead grid on *Ariphron* sp. patrol flight.

The effects of overhead shading on the flight of pollinators along existing patrol paths was tested by suspending two lengths of 2×1 m wooden garden lattice in line across minor tracks used by *Ariphron* sp. and other thynnines as patrol routes (Plates 11 and 12). The lattice reduced light transmission by 60 percent. It was suspended at three different heights (1.5, 0.6 and 0.2 m) at two locations.

Location 1 was on the north side of the gully to the north of the pumping station where a gridded roadway is proposed to be built above an existing *Ariphron* sp. flyway. This site had only a low *Ariphron* sp. population, but a larger population of another patrolling thynnine, *Thynnoides* near *senilis* was present and also monitored. Location 2 had a high *Ariphron* sp. population and was located on the minor track to the south west of the main JBLO colony beside the existing Leisure Centre access road.

Data recorded for both wasp species were the numbers flying below or over the lattice, and the numbers turning back during a five (Location 1) or ten (Location 2) minute observation period at each height. Limited time permitted only one replicate to be conducted at each site, except that two replicates were conducted for the 20 cm height at Location 1. The experiments were conducted on 15 November 2004.

Evaluation of the effects of a gridded barrier on *Ariphron* sp. patrol flight.

The responses of patrolling male *Ariphron* sp. and a second thynnine, *Thynnoides* near *senilis*, to a partial barrier erected across their flight path was tested using 4 m long by 1 m high wooden garden lattice, that had square openings with sides of approximately 6.75 cm. One end of the barrier was closed by placing it into dense shrubs beside the track, while the other end was open. Two replicates were run for 10 minutes each on 14 November 2004, during which the numbers of wasps going through the barrier, turning back or going around the open end of the barrier were recorded. The experiment was conducted at Location 1 described in the last section.

Phenology of *Prasophyllum affine*

Data on the phenology of flowering in the main JBLO population adjacent to the Leisure Centre access drive was collected on three occasions (4, 8 and 14 November). The numbers of unopened buds, open flowers and closed flowers were recorded for each plant.

Pollination levels in *Prasophyllum affine*.

Inflorescences of 72 individually numbered *P. affine* plants at the Vincentia main site were monitored on 4, 8 and 14 November to determine the levels of effective pollinator visitation to flowers and pollination rates. Each flower was examined on each inflorescence using a 10× hand lens. For each flower the presence or absence of the pollinarium, and whether the stigma had been pollinated, was recorded. Flowers were recorded as being pollinated irrespective of the amount of pollen deposited. Most commonly only trace amounts of pollen were placed on the stigma. Two categories for the amount of deposited pollen were recorded, trace or large. Removal of the pollinarium and pollination of the stigma both indicate a successful visit by a pollinator. Also recorded were whether the pollinaria had ‘sprung’ (Bower, 2002) and whether this had resulted in self-pollination. Factors that may result in loss of functional flowers, such as herbivory by caterpillars and webbing by spiders or caterpillars, were also recorded, but are not reported here.

Timing

The main part of the study was conducted in the first two weeks of November 2004 (3-15 Nov.). After JBLO flowering finished, supplementary observations for additional nectar sources for *Ariphron* sp. and Pepsinae were made on 3-4, 18-19 and 30-31 December, 2004.

RESULTS

Nectar sources utilised by pollinators of *Prasophyllum affine*

Some 5356 individual insects were recorded while feeding on the flowers of shrubs during 24.75 hours of formal blossom searches at Vincentia (Table 1). Additional opportunistic observations of pollinators were also made while moving around the site and in the course of other work. Some 62 records were of *Ariphron* sp., the dominant local pollinator of *Prasophyllum affine*. Other pollinators of *P. affine*, including Hatchet Wasps (Evaniidae), Spider Wasps (Pepsinae) and a Digger Wasp (*Cerceris* sp.) were also recorded, but in lower numbers, 10, 4 and 1 record, respectively. The pollinators were found on three shrub species (Table 1), Yellow Teatree, *Leptospermum polygalifolium* (Plate 1), a prickly teatree, *Leptospermum continentale* (Plate 3) and Dagger Hakea, *Hakea teretifolia* (Plate 4), and Port Jackson Mallee, *Eucalyptus obstans* (Plate 2). Golden Ants (*Polyrachis* spp.), a minor pollinator of *P. affine*, were relatively abundant (a total of 434 records) on the flowers of some shrubs, particularly *H. teretifolia* and *L. polygalifolium*, with 167 and 177 records, respectively (Table 1).

Phenology of *Ariphron* sp. food plants

Ariphron sp. was recorded on a succession of hosts between 10 November and 30 December (Figure 1). The first sightings were on Yellow Tea Tree, with 15 separate records from 10 to 12 November. After Yellow TeaTree, *Ariphron* sp. was recorded four times on Port Jackson Mallee on 14 and 15 November. Dagger Hakea was the main flowering plant on which *Ariphron* sp. was observed, accounting for 41 of the 62 records. Forty of these observations were made on 4 December when a single sighting was also made on flowers of *Leptospermum continentale*, on which a single pair was also seen the previous day. The final observation of *Ariphron* sp. was of a single unmated male on Dagger Hakea on 30 December.

While the positive sightings of *Ariphron* sp. are most important, the times and circumstances in which they were not found are also instructive. No *Ariphron* sp. were found in flower searches between 3 and 9 November, or on 18, 19 and 31 December. The reason for the absence of sightings before 10 November is unclear. Patrolling by *Ariphron* sp. males, and mating with females, was observed in the known breeding areas from November 3, and had evidently commenced some time earlier. In addition, Yellow Tea Tree blossom was abundant over large areas of the site, and it may have been that the density of nectar feeding *Ariphron* sp. was below the level of detection.

The first observations of *Ariphron* sp. on Yellow Tea Tree were made at the very end of the blossoming period for this species (Table 2), when the numbers of nectar secreting flowers were very low. It is likely that *Ariphron* sp. began to concentrate on the relatively few remaining suitable flowers towards the end of the flowering period and were easier to detect. It is notable that all 15 observations of *Ariphron* sp. on Yellow Tea Tree were made on the same small group of plants that were the latest flowering on the site. When, by 14 November, blossoming finished on these plants, *Ariphron* sp. was observed feeding on a small nearby group of Port Jackson Mallee trees that had just begun to flower. These trees would have provided a food source, albeit limited in quantity, for *Ariphron* sp. until late November.

At the same time that *Ariphron* sp. was switching from Yellow Tea Tree to Port Jackson Mallee, other nectar feeding insects were also switching to the mallee and to Tickbush, *Kunzea ambigua* (Plate 5), which was coming into full bloom, having commenced flowering around 7 November (Table 2). Tickbush is abundant in the south western parts of the site close to some of the known *Ariphron* sp. breeding areas. Despite this proximity, no *Ariphron* sp. were observed on Tickbush. The reasons for this are not known, but may relate to the effects of dilution on detectability. Flower phenology of *L. continentale* was approximately the same as for Tickbush (Table 2), but although distributed over most of the study area, its numbers and flower densities were much lower, making it less attractive to all nectar feeding insects except ants (Table 1). Nevertheless, two observations of pairs of *Ariphron* sp. were made on 3 and 4 December on *L. continentale*, one in an area where there had been previous observations of *Ariphron* sp. feeding on Yellow Tea Tree and Port Jackson Mallee between 10 and 15 November, and the other in a patch of Dagger Hakea being actively exploited by thynnines, including *Ariphron* sp.

Table 1. Matrix of nectarivorous insects and the major shrub and tree species on which they feed, Vincentia, New South Wales.
Data are the total numbers of each insect species or higher taxon observed in November and December, 2004.

Nectarivore Taxon				Species of Nectar Source						
Order	**Family**	**Species**	**Common name**	*Leptospermum juniperinum*	*Hakea teretifolia*	*Leptospermum polygalifolium*	*Leptospermum continentale*	*Eucalyptus obstans*	*Kunzea ambigua*	*Leptospermum trinervium*
Coleoptera			**Beetles**							
	Buprestidae		Jewel Beetles	2	2	15	3	2	19	
	Cantharidae	*Chauliognathus* sp.	Soldier Beetle	1468						
	Coccinellidae		Ladybird Beetles		2					
	Curculionidae		Weevils		6	2			3	
	Elateridae		Click Beetles		20	208		14	16	
	Mordellidae		Pin-tailed Beetles	4	7		3	1	5	
	Scarabaeidae		Nectar Scarabs	3	13	65	11	1	64	
	Other		Other Beetles		14	17		47	33	2
Hymenoptera			**Bees, wasps, ants**							
	Anthophoridae	*Exoneura* spp.	Reed Bees	2	27	1	3	3	27	2
	Apidae	*Apis mellifera*	Honeybee	3	312	273	1	61	13	4
		Trigona sp.	Sugarbag Bee					86	3	
	Other		Other native bees		4					
	Evaniidae		Hatchet Wasps		6		2	2		
	Formicidae		Ants	375	563	87	381	26	11	
		Myrmecia pilosula	Jumping Jack	4	48				1	
		Polyrachis spp.	Golden Ants		167	177	61	17	8	4
	Ichneumonidae	*Lissopimpla excelsa*			2					
	Mutillidae		Velvet Ant				1			
	Pepsinae		Spider Wasps		2	1		1		
	Pergidae		Sawflies			1			3	
	Philanthinae	*Cerceris* sp.	Digger Wasp		1					
	Thynninae	*Ariphron* sp.	A Flower Wasp		41	15	2	4		
		Asthenothynnus sp.	A Flower Wasp		80			1		
		Thynnoides aff. *senilis*	A Flower Wasp		3	3	1	10	8	
		Unknown 1	A Flower Wasp		25				1	
		Unknown 2	A Flower Wasp		2					
		Unknown 3	A Flower Wasp		1					
	Other		Other wasps		11	5	1	3	1	
Diptera			**Flies**							
	Syrphidae		Hoverflies		6	31		1	5	
	Other flies			9	151	10	6	8	17	3
Lepidoptera			**Butterflies and Moths**							
	Arctiidae		Day-flying Moths		7	1	2	1	21	1
	Lycaenidae		Blues		3					
Blattodea			**Cockroaches**							
	Blattellidae	*Balta* sp.			3					
Hemiptera			**Bugs**	3	3	2			1	
Total				1873	1529	908	477	293	260	16

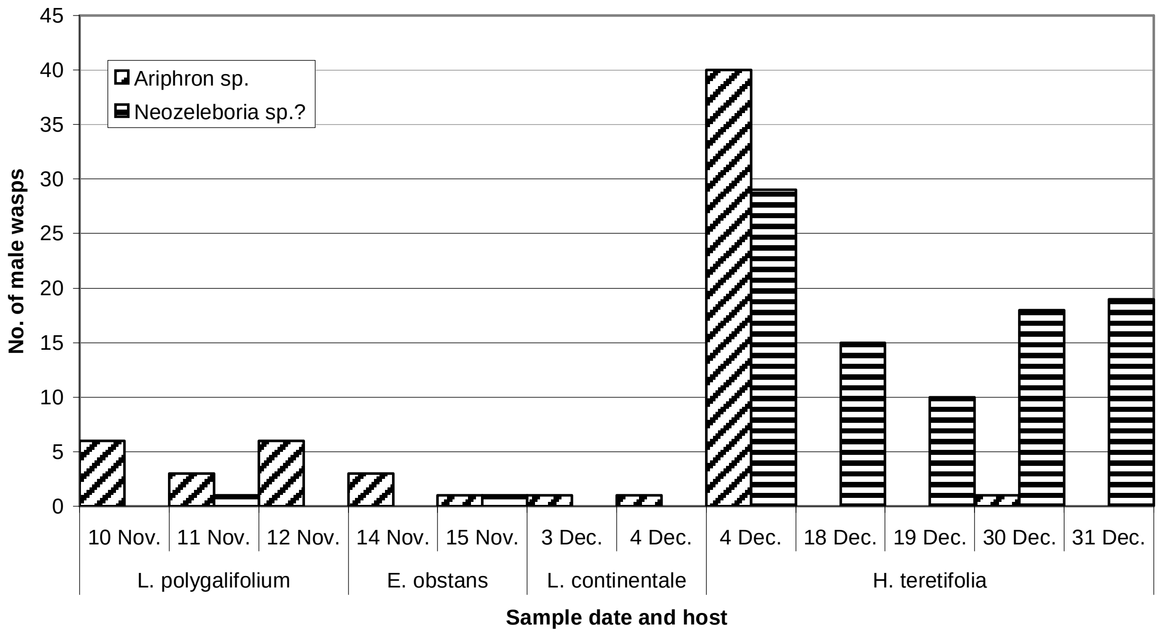

Figure 1. Observations of *Ariphron* sp. and *Neozeleboria* sp. on flowering shrubs and trees at Vincentia.

By 4 December flowering of Port Jackson Mallee and Tickbush was in decline (Table 2) in the south west of the study area. The focus for nectar feeding insects had shifted to Dagger Hakea, which was by then flowering abundantly in the sedgeland areas from north to south west of the main *P. affine* colony (Plate 6). Along with individual males and pairs of another thynnine wasp, tentatively identified as a *Neozeleboria* species, pairs and lone males of *Ariphron* sp. were relatively abundant on the Hakea flowers (Figure 1 and Table 1). It was noticeable that *Ariphron* sp. was not distributed randomly over the whole flowering Hakea population. Sightings were concentrated in two areas; a small area on the edge of the sedgeland west of the main *P. affine* colony, and a few plants on either side of the existing western powerline just to the south of the central watercourse. Both these sites are close to known *Ariphron* sp. breeding areas.

Two weeks later (18 and 19 December), Dagger Hakea was still flowering abundantly, but in different places, particularly around the edges of the sedgeland and into the adjoining heathland and forest areas, including within the main *P. affine* colony itself. By contrast to the situation two weeks earlier, no *Ariphron* sp. were found on the Hakea or anywhere else, including their known breeding areas. However, the *Neozeleboria* sp. was still abundant and remained so until at least the end of December (Figure 1), indicating the weather conditions were probably suitable for *Ariphron* sp. activity. These data suggest the *Ariphron* sp. season finished abruptly between 4 and 18 December. However, a single unpaired *Ariphron* sp. male was observed on Dagger Hakea on 30 December, indicating a few individuals may persist at least until the end of December.

Food plants of minor *Prasophyllum affine* pollinators

Spider Wasps of the family Pepsinae; Hatchet Wasps (Evaniidae) and Digger Wasps, *Cerceris* sp. (Philanthinae) are less common visitors to *Prasophyllum affine* than *Ariphron* sp. at Vincentia, but nevertheless contribute to pollination (Bower, 2002, 2004). They were also less common visitors to other nectar sources in the study area (Table 1), suggesting that their secondary status as pollinators of *P. affine* may simply be a reflection of low abundance. A total of ten Hatchet Wasps were observed on Dagger Hakea (6), *Leptospermum continentale* (2) and Port Jackson Mallee (2). Only four Spider Wasps were observed, two on Dagger Hakea, and one each on Yellow Tea Tree and Port Jackson Mallee. Only one Digger Wasp was seen and it was on Dagger Hakea. It is clear that all known pollinators of *P. affine* utilise the same alternative nectar sources.

Table 2. Flower phenology of the major nectar supplying shrub and tree species in November and December, 2004, Vincentia, NSW

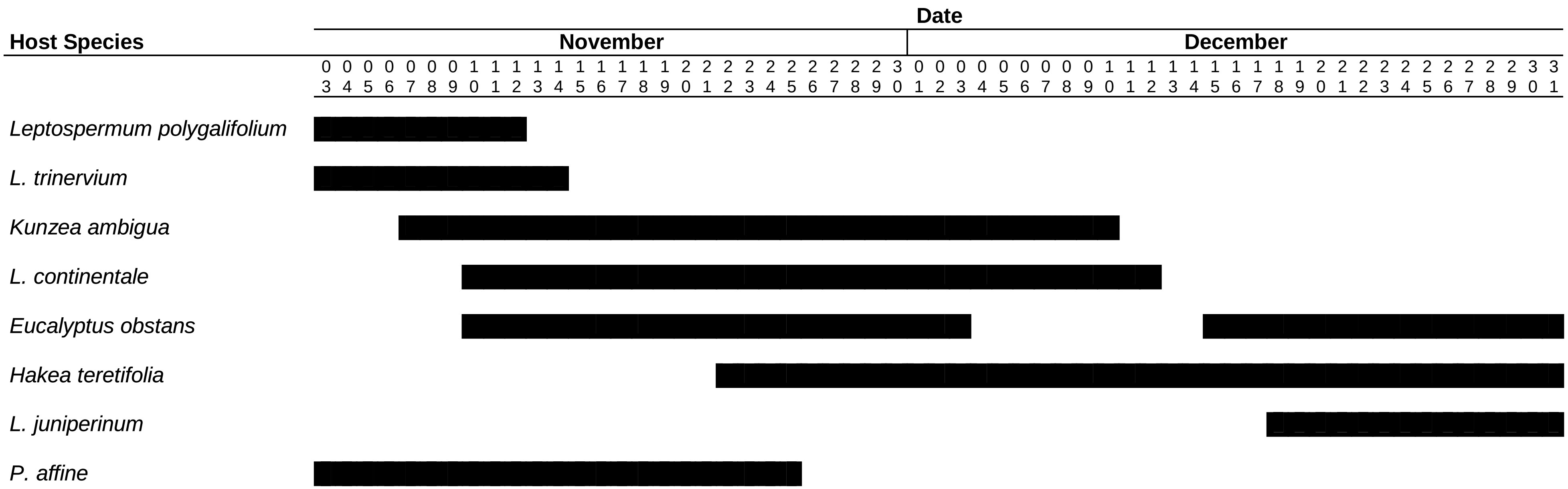

Plate 1. ***Leptospermum polygalifolium***

Plate 2. ***Eucalyptus obstans***

Plate 3. ***Leptospermum continentale***

Plate 4. ***Hakea teretifolia***

Plate 5. ***Kunzea ambigua***

Plate 6. ***Hakea teretifolia*** **flowering in the sedgeland on 5 December 2004.**

Feeding behaviour of *Ariphron* sp.

The great majority of observations of *Ariphron* sp. at nectar sources were of pairs (55 out of 62), consisting of a winged male and flightless female in copula. The remaining seven observations were of single feeding males.

The manner in which thynnine males assist females in copula to feed varies greatly among genera (Given, 1954; Alcock, 1981) and is worth recording here for *Ariphron*. Like all thynnines, the female is attached to the male by the genitalia and carried behind him in flight and when walking. The position adopted by attached females varies among genera; some keep their bodies straight, others are folded, with their head and thorax either above or below their abdomens. In the case of *Ariphron*, the female folds her head and thorax above the abdomen (Plate 7).

Male *Ariphron* sp. arriving in copula at a flowering Yellow Tea Tree, walk or fly to a flower and stand on the upper parts of the stamens. The male then bends his head down through the centre of the staminal ring and tests for the presence of nectar in the cup of the hypanthium. If nectar is present, the head is lifted out and the abdomen with female is bent between between the base of the stamens. The female unfolds herself, and must rotate her abdomen on the male genitalia to assume the feeding position below the male (Plate 8). She then takes hold of the substrate with her legs and drinks the nectar for about 5 to 10 seconds. When the female has finished she lets go, may wriggle and bend, and the male retracts her through the stamens. The male may then move to a new position on the same flower or go to a new flower. Up to three positions were used per flower.

A similar procedure was used on Dagger Hakea flowers, except that only one position was used per flower due to the smaller size of the nectar pool at the base (Plates 9 and 10).

Feeding areas and feeding flight distances of *Ariphron* sp.

A mark and recapture experiment on *Ariphron* sp. was conducted between 3 and 12 November. Some 68 male *Ariphron* sp. from four different breeding areas were individually marked. The experiment was not successful; only one male was recaptured, in the breeding area in which it was marked, and no marked males were observed feeding on a nectar source. Direct measures of male movement from breeding areas to food plants are therefore unavailable. However, it is possible to estimate the minimum likely distances males and pairs must have moved between known breeding areas and food sources, as follows.

Observations of feeding by *Ariphron* sp. males and pairs were highly clustered. Feeding observations were limited to three small patches, which seemed to be highly favoured. All *Ariphron* sp. feeding observations on Yellow Tea Tree between 10 and 12 November, 15 in total, were on the same late flowering shrub, located 58 m from the edge of the nearest known breeding colony and 80 m from the farthest extremity. Similarly, observations of feeding by *Ariphron* sp. on Port Jackson Mallee (14 and 15 November) all occurred in a small early-flowering patch immediately adjacent to the same Yellow Tea Tree bush, after it had finished flowering. Interestingly, one of only two observations of feeding by *Ariphron* sp. on *Leptospermum continentale* was made in the same patch on 3 December as Port Jackson Mallee flowering was finishing.

There were two quite separate clusters of *Ariphron* sp. feeding observations on Dagger Hakea, both distant from the above cluster on Yellow Tea Tree, Port Jackson Mallee and *L. continentale*. Nevertheless, both Hakea clusters were close to known *Ariphron* sp. breeding areas, and within 40 and 50 m at the closest edges and up to 70 and 110 m at the farthest extremities.

Plate 7. ***Ariphron*** **sp. male with female in copula on** ***Hakea teretifolia*****.**

Plate 8. Female ***Ariphron*** **sp. feeding on nectar in a** ***Leptospermum polygalifolium*** **flower.**

Plate 9. Female *Ariphron* sp. feeding on nectar in a *Hakea teretifolia* flower.

Plate 10. Female *Ariphron* sp. feeding on nectar in a *Hakea teretifolia* flower.

Relative importance of disturbed and undisturbed habitat for the breeding of *Ariphron* sp.

A series of transects was established to test for *Ariphron* sp. activity in undisturbed versus disturbed habitat, following observations in 2003 that suggested *Ariphron* sp. favours areas of minor disturbance for breeding. Because of the difficulty of observing *Ariphron* sp. flight over undisturbed habitat versus bare soil, the transect observations in 2004 depended on late afternoon or early morning sunlight reflected from the insects wings to detect them in areas with dense groundcover. Unfortunately, suitable light occurred on only one afternoon, allowing only one test to be performed; light to heavy cloud prevented observations on all other days. The results of this test are given in Table 3.

Table 3. Numbers of flying *Ariphron* sp. males observed per 5 minute observation period (one per site) (observations between 5.10 and 6.22 pm Eastern Daylight Saving Time)

Distance from origin (m)											
Distance	**-50**	**-40**	**-30**	**-20**	**-10**	**0**	**+10**	**+20**	**+30**	**+40**	**+50**
Time	6.17	6.11	6.05	5.59	5.53	5.10	5.16	5.22	5.29	5.34	5.40
Wasp nos.	0	0	0	14	0	24	1	0	0	0	0

The origin for this test was the closest known *Ariphron* sp. breeding area south west of the main *Prasophyllum affine* colony. The transect ran 50m north west of the origin into the sedgeland and 50 m south east to the edge of the dense heath and forest and parallel to section of the Leisure Centre access road that is perpendicular to Wool Road. Two five minute control counts were conducted at the origin half way through the test (5.47 pm) and at the end (6.30 pm), in which 48 and 3 patrolling movements of *Ariphron* sp. males were observed, respectively. These results show that between 5.47 and 6.30 pm there was a considerable decline in *Ariphron* sp. activity as evening approached, indicating the test was initiated a little too late for valid results throughout. Nevertheless, valid results were obtained for the first half of the test which show there was no *Ariphron* sp. patrol activity in the sedgeland. Interestingly, at the 20 m mark in the opposite direction there was a previously undetected area of activity with 14 movements recorded (Table 3). This activity was at the edge of a cleared area continuous with the breeding area at the origin. This result tends to reinforce the conclusion that *Ariphron* sp. breeding areas are mainly associated with minor disturbance. Beyond this disturbed area no further activity was seen, but the observations were made at a time when all *Ariphron* sp. activity was slowing down.

Responses of *Ariphron* sp. to barriers and overhead grids

Two simple experiments were conducted to determine the responses of patrolling males of *Ariphron* sp. and another thynnine species, *Thynnoides* near *senilis*, to gridded barriers, either across a flyway or at various heights above it.

Unfortunately, there were only low numbers of *Ariphron* sp. at Location 1, but the numbers of *T.* nr *senilis* were higher and may provide an indication of the general thynnine response to such barriers (Table 4).

Table 4. Behavioural responses of thynnine wasps to porous artificial barriers across male patrol routes, Location 1 (numbers of males).

Behaviour	*Ariphron* sp.		*Thynnoides* nr *senilis*	
	Rep 1	Rep. 2	Rep. 1	Rep. 2
Turn back	3	2	0	2
Go around	2	6	10	54
Go through	0	1	0	0

The dominant response by both thynnine species was to fly along the barrier and go around its open end. *Ariphron* sp. showed a greater tendency than *T.* nr. *senilis* to baulk in front of the barrier and turn back. Only one male, an *Ariphron* sp., negotiated its way through one of the 6.75 cm^2 openings in the lattice. None of the wasps went over the barrier; the one metre height was too great for these insects which normally fly just above the substrate, five centimetres or less for *Ariphron* sp. and 20 cm or less for the larger *T.* nr. *senilis*. That no wasps went over the barrier suggests that if the barrier had no open end, most wasps would have turned back, though possibly more would have found their way through the openings.

The responses of the two thynnine species to the overhead grid varied with the height of the grid and differed between species (Tables 5 and 6), as might be expected.

Table 5. Behavioural responses of Thynnine wasps to overhead grids suspended across a male patrol route at Location 1 (numbers of males).

Behaviour	***Ariphron* sp.**			***Thynnoides* nr *senilis***		
	Height of grid (m)					
	1.5	**0.6**	**0.2**	**1.5**	**0.6**	**0.2**
Go under	1	2	2	36	26	6
Turn back	0	0	1	6	11	8
Circle then under	0	0	0	2	1	1
Go over	0	0	1	0	2	27
Hit	0	0	0	0	1	1

Table 6. Behavioural responses of *Ariphron* sp. to overhead grids suspended across a male patrol route at Location 2 (numbers of males).

Behaviour	**Height of grid (m)**		
	1.5	**0.6**	**0.2**
Go under	15	16	17
Turn back	0	6	6
Circle then under	0	3	0
Go over	0	0	0
Hit	0	0	0

There was no effect on the behaviour of *Ariphron* sp. at a grid height of 1.5 m (Plate 11); all 16 observed males flew under the grid as they would have if it had not been there. By contrast, about 20 percent of *T.* nr. *senilis* either turned back or circled in front of the grid before passing underneath. At a grid height of 0.6 m (Plate 12) there was a change in the behaviour of *Ariphron* sp., such that about a third of individuals either turned back, or circled before going underneath. About two thirds of *T.* nr. *senilis* passed under the grid at 0.6 m, but the other third exhibited a variety of behaviours. Most simply turned back, but two opted to go over the top of the grid, one circled and went underneath and another, apparently confused, collided with the edge of the grid. At a height of 0.2 m, 70 percent of *Ariphron* sp. males went straight under the grid, the remainder turned back, except for a single male that went over the top. At 0.2 m 60 percent of *T.* nr. *senilis* flew over the grid, only 14 percent went under it, 19 percent turned back, one individual circled before going underneath and another hit the edge.

Although the data is fragmentary, lacking replication and sufficient numbers of observations, there are nevertheless consistent trends that allow preliminary conclusions to be drawn. It seems clear that a grid height of 1.5 m had no discernible influence on the behaviour of *Ariphron* sp. The experiment does not discount, therefore, that a gridded roadway at a height of 1.5 m will allow movement of *Ariphron* sp. below it. However, since the test grid was only one metre wide, it remains possible that a 10 or 15 m wide grid may inhibit movement by *Ariphron* sp., particularly as the data indicates that movement of another larger thynnine, *T.* nr. *senilis*, is likely to be inhibited under a gridded roadway. Therefore, it is not possible to conclude that *Ariphron* sp. males will be able to incorporate passage under a 1.5 m high gridded roadway as part of their normal patrol route.

Plate 11. Overhead grid set at 1.5 m above *Ariphron* sp. flyway, Location 1, Vincentia.

Plate 12. Overhead grid set at 0.6 m above *Ariphron* sp. flyway, Location 2, Vincentia.

Phenology of *Prasophyllum affine* in 2004

The phenology of *Prasophyllum affine* in the main Leisure Centre Drive colony in 2004 is shown on Figures 3 and 4.

Flowering of *P. affine* commenced some time before 4 November 2004, when eight flowering plants were found (Figure 2) with a total of over 100 open flowers (Figure 3). Several of these plants had been in flower for some time and were well ahead of the bulk of the population, which was still in bud. Over the next ten days most of the population came into bloom (Figures 2 and 3). By 14 November flowering had peaked and some pollinated flowers had closed (Figure 3).

Pollination of *Prasophyllum affine* in 2004

Visits by pollinators to flowers had commenced prior to 4 November by which time 15.2 pecent of open flowers had their pollinia removed and 5.7 percent had pollen on the stigma (Figure 4). As the number of open flowers increased, so did the percentage with pollinia removed from the anthers, suggesting that the orchid population became more attractive to insects as flower abundance increased. However, there was only a slight increase in the percentage of flowers pollinated to 8.4 percent on 8 and 14 November indicating a widening gap between the number of flowers with pollinia removed and the number pollinated (Figure 4). This suggests there was a high level of pollen wastage in 2004.

As in previous years, a small percentage of 'sprung' pollinia occurred in old flowers from which pollinia had not been removed by insects (Figure 4). A very small percentage (0.2%) of these had resulted in self-pollination by 14 November (Figure 4).

The initial period of the study in 2004 was completed before flowering of *P. affine* had finished. An attempt was therefore made on the subsequent short visits to assess the percentage of flowers that had successfully developed seed pods. This was problematic on 3 to 5 December because the seed pods were not mature enough for a reliable count. A count was therefore conducted on 19 December (Figure 5). It can be difficult in *Prasophyllum* species to distinguish fertilised ovaries from sterile ones as they may not greatly expand in size, particularly if the pollen load on the stigma is small (Jones, 1972; Peakall, 1987). Therefore, the putative seed pods were classified as small, medium and large. A problem with attempting to do this is that the size of flowers, and ovaries, varies considerably between plants. Individual plants with large ovaries will tend to develop larger seed pods than plants with small flowers. Therefore the classification of small, medium and large was made relative to flower or ovary size on the plant being assessed. This introduces a degree of subjectivity into the assessment. A further complication is that the seed pod in *Prasophyllum* does not split wide open along the ribs as in most other orchids, but opens via two quite small slits at the top of the ovary which may be difficult to see.

The seed pod assessment showed there were 7.7 percent large pods, 23.8 percent medium pods and 21 percent small pods, giving a total pollination level of 52.5 percent. Of these the small pod count is the most problematic; some of the small pods may have been doubtfully fertile. Therefore, 52.5 percent of flowers pollinated must be regarded as a maximum estimate. However, most of the flowers observed to be pollinated between 4 and 14 November had received only small pollen loads, which would result in only small or medium sized seed pods being formed.

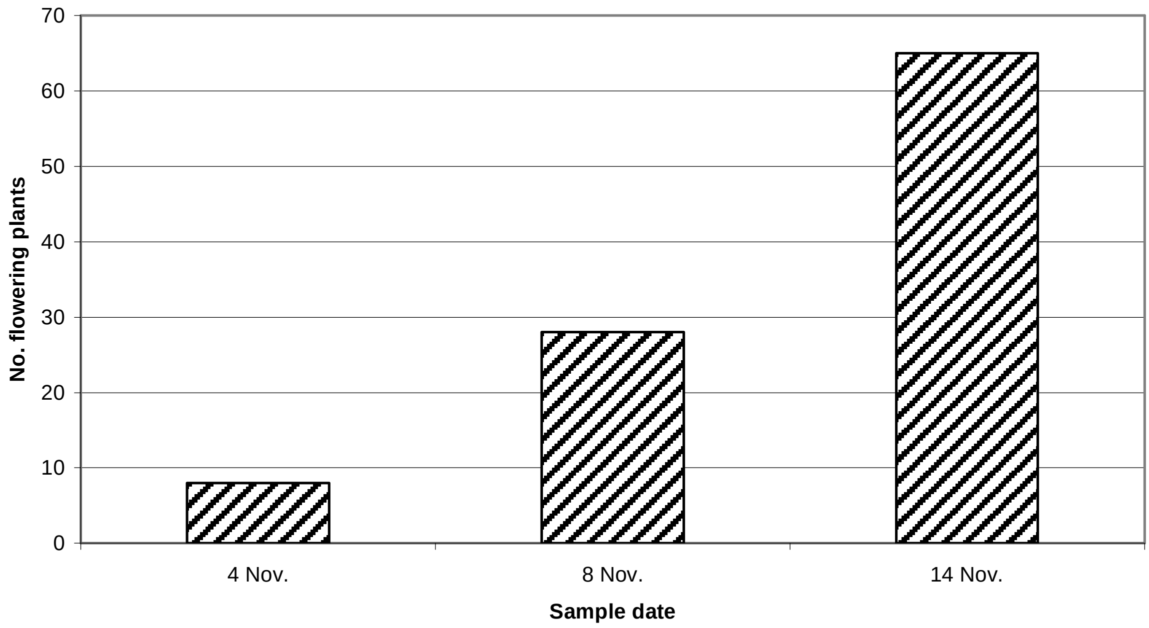

Figure 2. Number of plants of *Prasophyllum affine* with open flowers at the main Vincentia Leisure Centre Drive colony in November 2004.

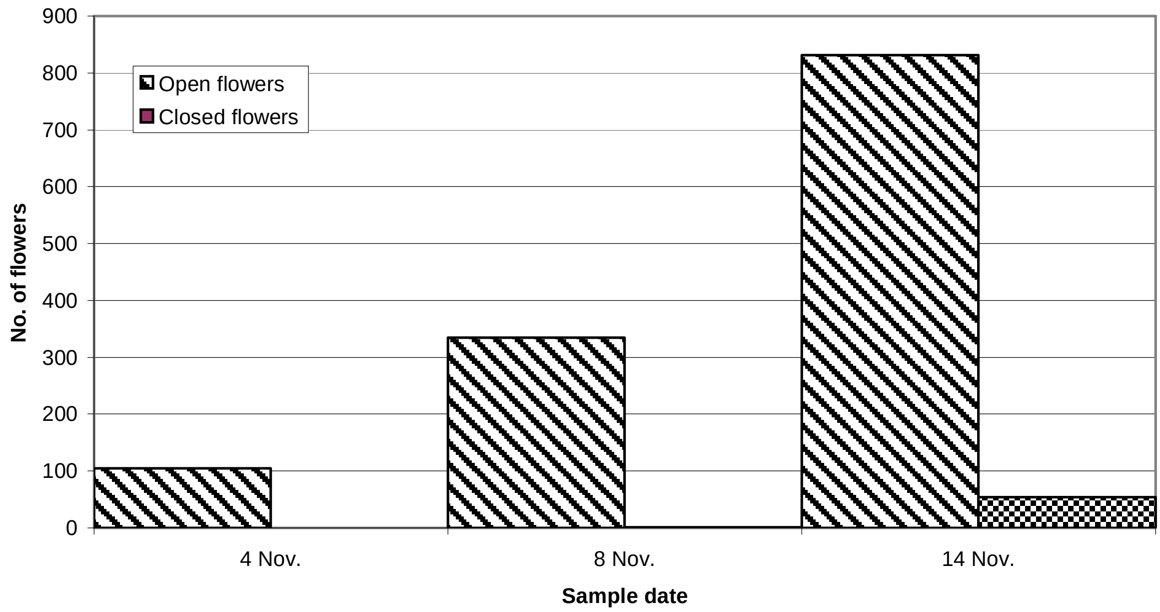

Figure 3. Total numbers of open and closed flowers of *Prasophyllum affine* at the main Vincentia Leisure Centre Drive colony in November 2004.

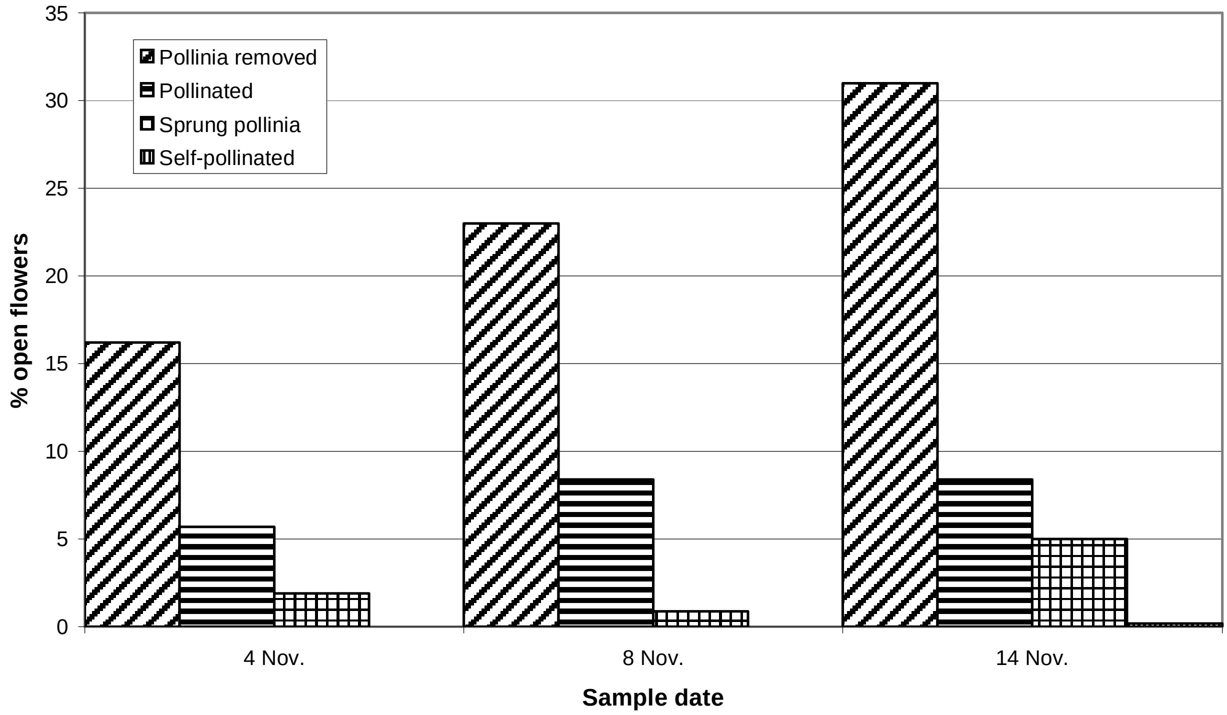

Figure 4. Pollination percentages for open flowers of *Prasophyllum affine* at the main Vincentia Leisure Centre Drive colony in November 2004.

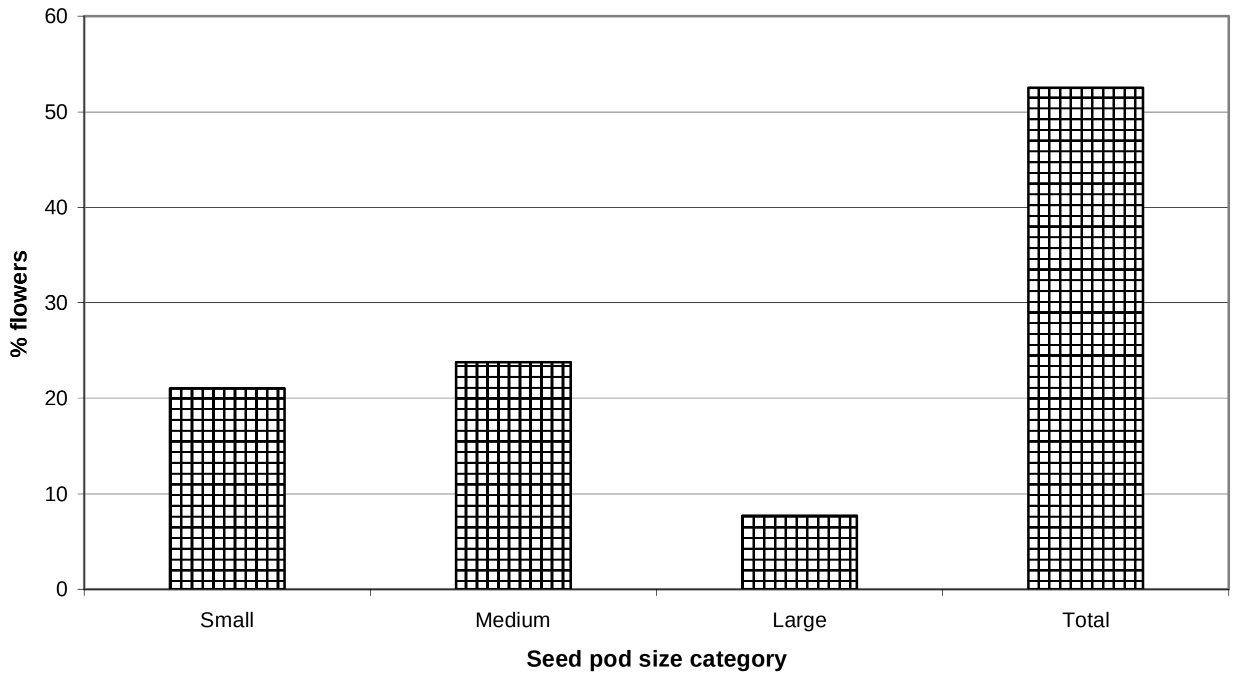

Figure 5. Percentages of total *Prasophyllum affine* flowers that developed small, medium and large seed pods in the main Vincentia Leisure Centre Drive colony in 2004.

DISCUSSION

Pollinator guild / pollination syndrome

The flowers of the shrub and tree species visited by *Ariphron* sp. and the minor pollinators of *Prasophyllum affine* were also visited by a wide range of other insect species from several orders and many families (Table 1). In general, a similar diversity of insect species was present on each of the nectar providing plants, although the species composition of visitors changed somewhat through time, as would be expected. The dominant orders represented were the Hymenoptera, Coleoptera and Diptera, in that order. These orders are generally the dominant blossom visitors to members of the plant family Myrtaceae, to which *Leptospermum, Kunzea* and *Eucalyptus* belong (Hingston and Potts, 1998; hingston and McQuillan, 2000; Hingston *et al.*, 2004). The six myrtaceous species on which flower visitors were observed in this survey all have white, strongly perfumed flowers with open nectar filled cups that are easily accessible to unspecialised nectar feeding insect species. It is clear that *Hakea teretifolia*, despite the very different architecture of its proteaceous flowers, belongs to the same pollination syndrome. *H. teretifolia* has dense, highly perfumed spikes of white flowers with abundant, easily accessible nectar for non-specialised insects. Since all the pollinators previously recorded for *P. affine* were part of the guild of visitors to the six myrtaceous and one proteaceous members of this generalised pollinator syndrome, it is clear that *P. affine* also represents this generalised insect pollination syndrome.

In view of the above discussion regarding the pollination syndrome, it seems likely that *Ariphron* sp. may also be able to utilise *Leptospermum juniperinum*, *L. trinervium* and *Kunzea ambigua*. This is most likely to be true for *K. ambigua*, since it was attractive to two other thynnine species (Table 1). *L. trinervium* is possibly atypical in that very few insects were observed on its flowers (Table 1), despite a large search effort. In general, it did not seem to be very attractive to nectar feeding insects, the reasons(s) for which are unknown. However, the plants were generally scattered and most carried only low densities of blossom. *L. juniperinum*, on the other hand, was widespread and abundant in the sedgeland areas, but was only just beginning to flower in late December amidst large volumes of Dagger Hakea blossom. However, even in early bloom it was highly attractive to some insects, notably ants and a localised concentration of Soldier Beetles (Table 1). It is likely that, as Dagger Hakea flowering declined in early January, the pool of generalised nectar feeding insects would switch from Hakea to *L. juniperinum*. Observations in this study concluded before this transition occurred, so although likely, it is hypothetical. However, in 2004, flowering of *L. juniperinum* occurred after the *Ariphron* sp. flight period was over, and hence was of no importance for this wasp in that year.

Distribution of nectar sources in space and time

Table 2 shows the phenology of the shrubs and mallee that are the dominant nectar sources for generalised nectar feeding insects at Vincentia. Table 2 shows a potentially continuous nectar supply during the observation period from early November to late December. Nectar availability would have extended longer than this, both at the beginning and end. The study commenced towards the end of Yellow Tea Tree (*Leptospermum polygalifolium*) flowering, which is likely to have extended from at least early October and possibly mid September. Many Yellow Tea Tree patches had long finished flowering in early November, particularly those in the open sedgeland areas, where flowering of most species seems to begin. Shrubs in the sedgeland are less shaded than in the heath and forest areas and daytime temperatures are likely to be warmer, thereby promoting earlier flowering. This was obvious during the study for Dagger Wattle (*Hakea teretifolia*) which flowered two weeks earlier in the sedgeland than in the surrounding dense heaths and forests. For each species there appears to be a wave of flowering across the site which radiates out from the sedgeland and finishes in the forests. Species distributed across the whole site may have individuals in flower for two months or more, for example Dagger Hakea and Port Jackson Mallee (*Eucalyptus obstans*) (Table 2). Since Yellow Tea Tree is also distributed throughout the study area, it is likely to have a two month flowering period from about mid September to mid November. Species with shorter flowering periods in Table 2, Tickbush (*Kunzea ambigua*) and *Leptospermum continentale*, were less common and tended to be confined to limited areas; in particular, they were absent from the sedgeland. Port Jackson Mallee is shown to have a broken flowering period in Table 2 due to patchiness in its distribution and a marked difference in flowering time between patches.

The two most important food sources for generalised nectar feeding insects at Vincentia in the September to December period are Yellow Tea Tree and Dagger Hakea. Yellow Tea Tree dominates in mid to late spring and Dagger Hakea in late spring to early summer. Both species are very widespread across the site and flower prolifically. In 2004, there was a short period in mid November when nectar supplies were relatively low between the end of Yellow Tea Tree and commencement of Dagger Hakea flowering (Table 2). This period was filled by Tickbush, Port Jackson Mallee and *Leptospermum continentale*. However, these species, particularly the Port Jackson Mallee and Tickbush, have more localised distributions, and/or are much lower in abundance (*L. continentale*) than Yellow Tea Tree and Dagger Hakea.

Feeding flight distances of *Ariphron* sp. and feeding areas

Mark and recapture experiments to determine flight distances between breeding areas and nectar sources for *Ariphron* sp. were not successful. However, minimum flight distances were inferred by measuring the distances between food plants on which males were observed to be feeding and the nearest known breeding area. This gave minimum flight distances ranging from 40 to 60 m.

Apart from a small population of *Ariphron* sp. known to breed within the area of the main *Prasophyllum affine* colony beside the Leisure Centre access road, the closest known *Ariphron* sp. breeding area is about 90 m to south west (see Figure 1 in Bower, 2004). This is also one of the largest breeding populations in the study area and is likely to be the dominant source of pollinators for the main *P. affine* colony. Two of the three observed *Ariphron* sp. feeding clusters were close to this breeding area, and suggests the wasps were flying at least 40 m, and up to 110m, in opposite directions at different times to obtain nectar. This *Ariphron* sp. breeding area and both feeding clusters are well within the designated environmental protection zone for the development. These data should be considered in conjunction with mark and recapture results obtained in 2003 (Bower, 2004), which showed that two *Ariphron* sp. males found on *P. affine* inflorescences in the main colony had travelled at least 94 and 213 m in a straight line from the breeding areas where they were marked.

Breeding areas

Attempts to quantitatively assess the relative importance of minor disturbance areas versus undisturbed habitat for the breeding of *Ariphron* sp. were thwarted by unfavourable overcast weather conditions. Only one trial could be conducted and it showed that *Ariphron* sp. was absent from the undisturbed areas examined. However, the data are insufficient to conclude that *Ariphron* sp. does not breed in undisturbed habitat. Evidence of small scale breeding in grassland areas was obtained in 2003 (Bower, 2004), when male patrolling and pickup of females was observed in the main *Prasophyllum affine* colony area. Nevertheless, the available evidence suggests that most *Ariphron* sp. breeding activity takes place along the edges of minor tracks where populations of host beetle larvae may be higher (Bower, 2004).

Overhead grids

The effects of partial shading of patrol routes by 1 m wide overhead grids showed there was no effect on the behaviour of *Ariphron* sp. males when the grid was placed 1.5 m above the ground. However, when lowered to 0.6 and 0.2 m the grid inhibited the movement of a third of patrolling males. The patrolling movements of 20 percent of *Thynnoides* near *senilis* males, a larger thynnine species, were inhibited by the grid at 1.5 m. While the data does not show an inhibitory effect on *Ariphron* sp. for a one metre wide grid at 1.5 m high, it does not allow conclusions about road width grids.

Phenology of *Prasophyllum affine*

Peak flowering of *Prasophyllum affine* was earlier in 2004 (14 November) than in 2003 (20 November) (Bower, 2004), but later than in 2001 (6 November) (Bower, 2002) indicating there is considerable variation in flowering phenology between years.

Pollination of *Prasophyllum affine*

The percentage of open flowers with pollinaria removed by insects at the peak of flowering was 31 percent in 2004, similar to 2001 (Bower, 2002), but lower than 2003 (49 %) (Bower, 2004). At the same time only 8.4 percent of open flowers had been pollinated in 2004, versus 21 percent in 2001 (Bower, 2002) and 35 percent in 2003 (Bower, 2004). The very low ratio of pollination of stigmas to removal of pollinaria from the anthers differs markedly from the previous years. The explanation may relate to the low populations of *Ariphron* sp. present at vincentia in 2004. This is shown by the low number of male wasps that were able to be marked in the mark and recapture work, only 68 in 2004 versus 187 in 2003 (Bower, 2004). The low numbers marked in 2004 reflect a genuinely lower population. Males were less obvious in all breeding colonies and were absent from some areas they had occupied in 2003. While the generally overcast weather in 2004 may have been partly responsible, it was obvious that numbers were much lower when conditions were favourable for wasp activity. It is therefore likely that *Ariphron* sp. was responsible for a much lower proportion of pollinaria removals from *Prasophyllum affine* in 2004 than in previous years. It is possible that the majority of pollinaria removals were by much larger species such as the Spider Wasps (Pepsinae), that can remove pollinaria from their faces manually. This suggests there may have been a much higher level of pollen wastage in 2004 than in previous years.

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