

**Biology and phenology of *Floracarus perrepae*, on its native host in Australia, Old World climbing fern, *Lygodium microphyllum***

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**Abstract.** The biology and phenology of the eriophyid mite, *Floracarus perrepae* Knihinicki and Boczek, a potential biological control agent of *Lygodium microphyllum* (Cav.) R. Br., was studied in its native range - Queensland, Australia. *Floracarus perrepae* forms leaf roll galls on the subpinnae of *L. microphyllum*. It has a simple biology, with females and males produced throughout the year. The population was female biased at 10.5 to 1. The immature development time was  $8.9 \pm 0.1$  days and  $7.0 \pm 0.1$  days; adult longevity was  $30.6 \pm 1.6$  days and  $19.4 \pm 1.2$  days and mean fecundity per female was  $54.5 \pm 3.2$  eggs and  $38.5 \pm 1.6$  eggs at 21°C and 26°C, all respectively. Field studies showed that the mite was active year round, with populations peaking when temperatures were cool and soil moisture levels were highest. Two species of predatory mites, *Tarsonemus* sp. and a species of Tydeidae, along with the pathogen *Hirsutella thompsonii*, had significant effects on all life stages of *F. perrepae*. Despite high levels of predators and the pathogen, *F. perrepae* caused consistent damage to *L. microphyllum* at all the field sites over the entire two years of the study.

**Key words:** Biological control, invasive species, Florida Everglades, developmental parameters, plant - feeding mites, native range studies

## Introduction

*Lygodium microphyllum* (Cav.) R. Br. (Lygodiaceae, Pteridophyta), Old World climbing fern, is native to the wet tropics and subtropics of Africa, Australasia, Asia, and Oceania and is typically found growing in swamp conditions, twining up the trunks of trees (Pemberton, 1998). In southern Florida, *Lygodium microphyllum* is an aggressive invasive weed, and because it had become so widespread and damaging (Pemberton and Ferriter, 1998), a biological control program was initiated and surveys for potential agents in Australia and South Asia were conducted (Goolsby *et al.*, 2003). The eriophyid mite, *Floracarus perrepa* Knihinicki and Boczek, was the most widely distributed herbivore and several distinct genotypes were identified (Goolsby *et al.*, 2004a). Throughout its native distribution in Australia and Asia, *F. perrepa* causes significant damage to *L. microphyllum*. *Lygodium microphyllum* grows as a climbing vine, with each shoot (leaf) composed of pinnae (leaflets) and subpinnae (subleaflets). Feeding by the adults and immatures on the subpinnae causes formation of leaf roll galls (*sensu* Westphal, 1992), leading to necrosis and premature defoliation of *L. microphyllum* pinnae, and the gradual debilitation of the plant. This impact on *L. microphyllum* was measured in outdoor chemical exclusion trials, and *F. perrepa* was found to have a significant impact on biomass production (Goolsby *et al.*, 2004b). Based on its narrow field host-range and significant impact on *L. microphyllum*, *F. perrepa* was prioritized for further evaluation as a biological control agent (Goolsby *et al.*, 2003).

Although biological control of weeds is dominated by the use of insects as agents, several mite species have been utilized (Julien and Griffiths, 1999). The entire superfamily of Eriophyoidea is characterized by species with narrow host-ranges on perennial hosts (Cromroy, 1978; Lindquist and Oldfield, 1996), making them ideal candidates for biological control of weeds (Andres, 1982; Rosenthal, 1996; Briese and Cullen, 2001; Cullen and Briese, 2001). Several species of Eriophyidae have been used in biological control programs targeting weeds: *Aceria acroptiloni* Kovalev and Shevchenko (Kovalev, 1973); *Aceria chondrillae* (G. Can.) (Sobhian and Andres, 1978; Cullen *et al.*, 1982; Boldt and Sobhian, 1993); *Aceria malherbae* Nuzzaci (McClay *et al.*, 1999; Michels *et al.*, 2000); *Aculus hyperici* (Liro) (Willis *et al.*, 1995; Mahr *et al.*, 1997); *Phyllocoptes nevadensis* Roivainen (Littlefield and Sobhian, 2000) and *Phyllocoptes fructiphilus* Keifer (Amrine, 1996). However, there are few studies

that document the phenology of an eriophyid mite in its native range prior to its release in its adventive range as a biological control agent. Phenological studies in the native range and biological studies under different conditions can be useful in determining the effect of climate on mite populations. These types of studies allow one to predict if climatic factors will limit the potential geographic range for introduction. Biological studies of the mite may also be useful in developing rearing methods, meaningful host-range tests, and appropriate release methods. Knihinicki and Boczek (2002) identified and described *F. perrepae*, but there is no information available on either the biology or phenology of this mite. Based on the successful record of eriophyoid mites in biological control and the apparent potential of *F. perrepae* as a biological control agent of *L. microphyllum*, studies were initiated to investigate its biology and phenology.

## **Materials and Methods**

### *Biological Studies*

Laboratory research was conducted at the USDA-ARS, Australian Biological Control Laboratory, CSIRO Long Pocket Laboratories, Indooroopilly, Queensland (QLD), Australia, between October 2000 and September 2002. Greenhouse grown *L. microphyllum* infested with *F. perrepae* were used in the study. *Floracarus perrepae* were collected from leaf rolls on the subpinnae of *L. microphyllum* pinnae. Both the plant and mite were originally collected from along Carbrook Creek, near Logan, QLD. Sporelings of *L. microphyllum* were used in the study to allow for small-scale observation of the mites on living plants. Spores of *L. microphyllum* were sown in a soil-less medium (Jones, 1987) and later transplanted into pots measuring 2.3 cm height X 1.7 cm diameter. The sporeling plants had 2-4 true leaves (leaf = pinna) and were 1-3 cm tall. Some pots had more than one sporeling, but they generally contained one individual. Five pots were placed into a small plastic container with a perforated floor to allow the pots to absorb water. Six small containers were placed in a large plastic container that was filled with water to 1 cm depth. Each large container was wrapped in white organza to prevent contamination and a single layer of paper towel was placed over the container to increase shading. The experiments were

carried out consecutively in a growth cabinet at 21°C and 26°C, at 70% RH and under artificial light (15,000 lux) with a 14:10 hour L:D cycle.

Several experiments were conducted to study the biology of *F. perrepa*. In each experiment a replicate consisted of mites that settled on the open leaf surface of sporelings. This method was used to allow for clear visual inspection of behaviour. Many *F. perrepa* formed leaf rolls but these replicates could not be used because their life stages could not be observed without damage to them or the sporeling. Mites were observed with a dissecting microscope at 50X and 100X.

To develop rearing methods for *F. perrepa*, observe their behaviour, and study leaf roll induction, 10 to 20 adults were transferred to each sporeling replicate and observed daily.

To determine development times from egg to adult, 10 adults were transferred to the each sporeling. After they had laid 1 or 2 eggs in total, the adult females were removed from the subpinnae and transferred to a clean sporeling to produce separate replicates. Observations of immature stages were made once and sometimes twice daily on each replicate. The total numbers of egg to adult observations were 35 at 21°C and 18 at 26°C. Analysis of variance was used to test for differences between development times for all stages at the two temperatures. Approximate development rate and developmental zeros were estimated from the data at the two temperatures.

To determine the periods of preoviposition, oviposition, postoviposition, adult longevity and total mean egg production of *F. perrepa*, in each replicate, two newly emerged adults from the development time studies were transferred to the subpinnae where males had deposited spermatophores. As only one female was required, if they both settled on the subpinna, one was removed. Nearly all observations were made daily but when the time interval between observations was greater than one day, the eggs were averaged between the days. Daily counts were made of newly emerged adults to verify prior egg deposition and determine mortality from egg to adult stage. Age-specific fecundity and egg mortality were determined for 12 females at 21°C and 13 at 26°C, respectively. Nymphal and larval mortality were determined from the development data and combined with egg mortality to give the proportion of immatures which did not survive. Based on these studies, the intrinsic rate of increase and the population doubling time were determined for both temperatures, using the methods of Southwood (1978).

Sex ratio was determined from 197 adults collected from field samples and newly emerged adults from the oviposition studies. After storage in 70% ethyl alcohol, working slides of mites were prepared using lactic acid. Slides were then placed on a hot plate for 20 minutes on low heat. The mites were then observed with a microscope at 400X and 1000X magnification using immersion oil.

### *Field Phenological Studies*

Four sites in southeast Queensland were selected for study of the phenology of *F. perrepae* on its host, *L. microphyllum*. Two sites were selected on Bribie Island, QLD: Gallagher's Point (27° 01.17' S, 153° 06.53' E) and McMahon Road (27° 04.33' S, 153° 10.55' E). The other sites were approximately 70 km south of Bribie Island near Logan, QLD: Carbrook Creek (27° 41.30' S, 153° 16.23' E) and Lagoon Road (27° 40.01' S, 153° 16.03' E). The sites are typical habitats within the native range of both *L. microphyllum* and *F. perrepae* in subtropical, eastern Australia. At the sites, the fern was typically found climbing up the trunks of trees, reaching up to 10 m, and with many yellowish-green, fertile or sterile pinnae branching off the main stem. Each pinna consists of 6-12 paired subpinnae, which are the smallest leaf unit. Fertile subpinnae are fringed with lobes of sporangia, with sterile subpinnae having a smooth outer margin. *Floracarus perrepae* was observed to feed on and cause leaf rolls on the fertile subpinnae, but they did not appear to prefer this leaf form. Therefore, the field phenological studies were based on counts of sterile subpinnae. All the sites are seasonally inundated with water, which often persists during the summer months. The stand of *L. microphyllum* at Lagoon Road was regrowth following a bush fire in 1998.

At the Lagoon Road and Carbrook Creek sites, samples were taken each month between February 2001 and February 2003. On Bribie Island, twenty-five samples were taken from Gallagher's Point from March 2001 to February 2003. At the McMahon Road site only 12 samples were taken as the site was destroyed by fire, and mites did not appear on the regrowth of *L. microphyllum* for the remainder of the two-year study. Each month, 50 newly expanded sterile pinnae were hand collected at each site from along a transect and taken to the laboratory. The numbers of rolled and unrolled subpinnae were then counted for each pinna. This count provided a measure of the proportion of infested subpinnae (leaf rolls), or mite damage, at each location.

From these subsamples, 30 infested subpinnae were removed to count the numbers and stages of *F. perrepae* within each leaf roll. All associated predatory mites within each leaf roll were also counted and identified and the presence or absence of the mite associated pathogen, *Hirsutella thompsonii* Fisher, was assessed. Observation and counts of mites and pathogens were made at 100X with the use of a dissecting microscope. Representative adult *F. perrepae* and the associated predator species were removed and placed in 70% ethanol for identification and sex ratio studies. *Floracarus perrepae* specimens were vouchered with New South Wales Agriculture, Orange, New South Wales.

The monthly estimates of the proportion of leaf rolls and the numbers of mites per leaf roll at the four sites were analyzed to determine which biotic and abiotic factors may be having the greatest influence. The biotic factors were predatory mite species, pathogen and sites. The abiotic factors were maximum and minimum temperature, rainfall, RH at 9 am, RH at 3 pm, radiation, soil moisture (calculated using a simple model developed by Fitzpatrick and Nix, 1969), saturation deficit, and the product of the saturation deficit with maximum temperature. Weather data was provided by the Queensland Department of Natural Resources. The weather variables used in the analysis were means for one week, two weeks, and one month prior to the sampling date, to determine the most relevant period. Due to their proximity to one another, the same soil moisture value was used for Lagoon Road and Carbrook Creek and another value was calculated for Gallagher's Point and McMahon Road. Density of *F. perrepae* and predatory mites were calculated by multiplying the proportion of leaf rolls by the mean number of mites per leaf roll. Pathogen density was calculated by multiplying the proportion of leaf rolls by the proportion of leaf rolls with infected mites. The density was not used as a dependent variable in the analyses of the biotic and abiotic factors when it was determined that the measures, mites per leaf roll and proportion of leaf rolls, were affected differently by the weather variables. A separate analysis of variance was done for both of these measures. The mites per leaf roll were further partitioned into numbers of adults, immatures and eggs, and analyzed in a similar analysis using a square root transformation.

The annual field intrinsic rate of increase for *F. perrepae* in southeast Queensland was estimated using the methods of Carey (1993) and Pratt *et al.* (2002). The period used in the estimate of intrinsic rate of increase was March 2001 to February 2002. A mean density for the four sites was calculated each month for

twelve months and these were used to calculate the mean finite rate of increase per month. This was raised to the 12<sup>th</sup> power to give the finite rate for the year, which is  $\lambda$ . The annual intrinsic rate of increase was then calculated as  $r = \ln(\lambda)$  (Pratt *et al.*, 2002).

## Results

### *Biological Studies*

*Floracarus perrepa* has a simple life cycle with no deuteroecy, i.e. there was only one type of female. Females and males were produced throughout the year. The mite commences life as an egg, passes through two immature stages, (a larva and a nymph), and finally emerges as an adult. A quiescent or resting stage occurs between the larva and nymph, the nymphochrysalis, and again between the nymph and adult, the imagochrysalis. Eggs were approximately 0.041mm in diameter. They were spherical and translucent and difficult to see when freshly laid. Within a few days of being laid, they changed to a creamy-white colour and became more visible. First eggs were generally male. The larvae were transparent. The nymphs were also transparent, as they were in the inactive stage but segmentation of nymphs was easily seen. Immatures were similar in appearance to adults which are 173-226  $\mu$ m long (Knihinicki and Boczek, 2002), but smaller in size. Males were similar to females, but slightly smaller and narrower. Newly emerged adults were cream in colour, changing to light-brown or a creamy-yellow colour in 6-7 days and slightly darker in 9-10 days. In 18-20 days, the adults had turned brown. The dorsal shield and posterior of the body were brown, although the rest of the body was more beige-brown coloured. All adults were still brown when they died, approximately one month after emerging.

The entire life cycle was completed within a period of 9-12 days. The life history parameters of *F. perrepa* are shown in Table 1. There were significant differences among the development times, oviposition periods and adult longevities for the two temperatures. The life table parameters also varied greatly between the two temperatures (Table 2). The development rates and developmental zeros, based on the two temperatures, were only approximate but it indicated that for this species, the developmental zero was less than 5°C. Daily egg development rate was 0.01126

(T-3.3), where T is temperature. Daily larva to adult development rate was 0.01316 (T-1.6). Daily total immature development rate was 0.00608 (T-2.6).

As in other eriophyoid mites, the male of this species produced spermatophores which they attached to the host plant by a curved stalk, and fertilization occurred when the female drew the spermatophore into the genital orifice. Fertilization occurred at the feeding sites – subpinnae including leaf rolls and growth points - where spermatophores were available. Females began reproducing shortly after shedding their nymphal skin and died shortly after completing reproduction (Table 1). Total immature period was 8.94 days and 7.03 days, and adult longevity was 30.58 days and 19.38 days at 21°C and 26°C, respectively. Proportional survival of *F. perrepae* females at 21°C and 26°C is shown in Figure 1. Females laid eggs at the feeding sites until the feeding sites began to dry out. If the feeding site was still green, newly moulted females stayed and laid eggs. Females also laid eggs between the first two newly emerging pinnae of the juvenile sporelings, which later resulted in the death of many sporelings because of feeding pressure on the growth points. The mean egg production was significantly different between the two temperatures, with  $54.50 \pm 3.18$  (n=12) and  $38.50 \pm 1.57$  (n=13) eggs laid at 21°C and 26°C, respectively. Mean egg lay per day is shown in Figure 2.

The host plant's tissue was very sensitive to feeding by *F. perrepae*. In response to feeding, the normal epidermal cells became significantly enlarged, causing the subpinna to roll. Mature epidermal cells become meristematic, greatly increasing their cytoplasmic content and metabolic activity to form the nutritive tissue of the roll (Freeman *et al.*, 2004). Mature subpinnae were not preferred for feeding. The majority of adults preferred to feed on young sterile subpinnae. Leaf rolls were commonly formed on the margins of the subpinnae, and infrequently, the entire margins of subpinnae were rolled. Because the mites fed on either surface of the subpinna, the leaf rolls developed either upward or downward, with 1-3 windings, and most commonly 2 or 3. A single female is capable of inducing a large leaf roll gall compared with the size of its body, and in 2-3 weeks the leaf roll became fully occupied by its offspring. The larvae, nymphs and adults continued to feed on the deformed tissue in the leaf rolls. As the leaf rolls dried as a result of continuous feeding, the adult mites migrated to other feeding sites where the cycle was repeated. The dried leaf rolls then abscised from the remainder of the subpinnae, which

eventually became necrotic and dried out. Very rarely, upward leaf curving was followed by the folding of the subpinnae back on itself. It was also noted that mite feeding did not always induce leaf rolls. Mites also fed on the tips of the newly emerged and rapidly expanding terminal growth. Between 20-25 days after infestation, there was significant damage and complete deformation of the tips of the sporeling shoots. At about 35 days after infestation, the tips of the shoots had dried out as a result of heavy feeding pressure. At that time, the population density was very low on the tips, as the mites had spread to other young subpinnae, stems and roots where they continued feeding, often causing sporeling deaths. 'Jumping' behaviour was commonly observed when mites were leaving the feeding sites. Adult mites stood erect with the aid of their anal suckers and rocked forwards and backwards before somersaulting through 360° in a forward direction.

Analysis of the duration of leaf roll formation showed that temperature had no significant effect on the time it took for the mites to form leaf rolls. The rolls took  $4.15 \pm 0.32$  days (n=13) and  $3.75 \pm 0.17$  (n=17) at 21°C and 26°C, respectively, to develop. The margins of some subpinnae began to roll one to two days after the mites had settled and began feeding. Some individuals took longer to settle, and in some cases did not form complete leaf rolls until 13 days after infestation.

The sex ratio of *F. perrepae* was female biased (91.37%). The males of *F. perrepae* were much less numerous than the females, but they produced large numbers of spermatophores. There was no significant difference in the proportion of females between field and laboratory samples.

### *Field Phenological Studies*

The mean numbers of adults and immature *F. perrepae*, the proportion of leaf rolls and mean densities of the mite at four field sites in southeast Queensland are presented in Table 3. The highest overall mean density was found at Carbrook Creek and the greatest proportion of leaf rolls was found at McMahon Road.

The density of *F. perrepae*, and its primary natural enemies (predatory mites and pathogen) along with temperature and soil moisture index are shown in Figure 3. For clarity only the data from one representative site (Lagoon Road) are shown in the figure. The average weekly temperature follows an annual pattern, seasonally

fluctuating between 15°C and 26°C. Soil moisture fluctuated, but was low over the two years due to the prevailing drought conditions experienced throughout eastern Australia. Soils reached saturated conditions 17 times during this study. Values of greater than one indicate soil moisture saturation with surface water runoff. Population fluctuations of *F. perrepae* did not follow a seasonal pattern and peaks in numbers were observed at various times throughout the year. A pathogenic fungus, *Hirsutella thompsoni*, was common during the winter months, and predatory mite populations increased following increases in *F. perrepae* numbers. Predatory mites were collected from leaf rolls and were observed feeding on the *F. perrepae* adults, immatures and eggs. The first four of the following six species were commonly encountered: *Tarsonemus* sp. (Tarsonemidae), tydeid sp. 1, tydeid sp. 2 (Tydeidae), *Agistemus* sp. (Stigmaeidae), and one species each of Cheyletidae and Ascidae. At some locations, syrphid larvae (Syrphidae) were observed feeding on *F. perrepae*.

The proportion of leaf rolls (=plant damage) is shown for the four field sites (Table 3). Of the biotic factors analyzed, only sites was significant. Soil moisture of the preceding month was the only abiotic variable that was significant in analysis of the proportion of leaf rolls [regression coefficient (b) = 0.265 se 0.080,  $F_{1,81} = 11.07$ ,  $P < 0.01$ ]. After accounting for the effect of soil moisture, there were still significant differences between field sites in the analysis ( $F_{3,81} = 3.01$ ,  $P < 0.05$ ) and these two factors explained 20% of the variation. The proportion of leaf rolls increased as moisture increased by the same amount at each site.

The mean numbers of *F. perrepae* adults, immatures and eggs per roll at the four sites are shown in Table 4, with Carbrook Creek having greater mean numbers of all life stages. There were no interactions between sites. The strongest influences on numbers of adults and immatures in the analysis were temperature, with numbers of *F. perrepae* decreasing as temperature increased, and density of tydeid sp. 2, which caused significant reductions in numbers of mites per roll.

*Adults.* In the analysis of adults per leaf roll, three factors: sites ( $F_{3,81} = 3.11$ ,  $P < 0.05$ ); tydeid sp. 2 ( $b = -0.716 \pm 0.203$ ,  $F_{1,81} = 13.05$ ,  $P < 0.001$ ); and mean minimum temperature of the preceding two weeks ( $b = -0.0637 \pm 0.0163$ ,  $F_{1,81} = 15.29$ ,  $P < 0.001$ ), explained one third of the variation. As there were no interactions between the sites and the other factors, the estimated regression coefficients are equal for all sites.

*Immatures* (larvae and nymphs). In the analysis of immatures per leaf roll, four factors: sites ( $F_{3,79} = 3.57$ ,  $P < 0.05$ ); tydeid sp. 2 ( $b = -0.873 \pm 0.279$ ,  $F_{1,79} = 3.57$ ,  $P < 0.05$ ); minimum temperature ( $b = -0.0388 \pm 0.023$ ,  $F_{1,79} = 3.50$ ,  $P < 0.06$ ) and tydeid sp. 1 ( $b = -1.488 \pm 0.731$ ,  $F_{1,79} = 4.14$ ,  $P < 0.05$ ) were all significant, explaining 25% of the variation. The effects on the immature population were not as strong as those observed for adults. This may be a result of the association of immatures with adults ( $r = 0.83$ ), as the above factors were not significant after adjusting for adults in the analysis.

*Eggs*. In the analysis of eggs per leaf roll, five factors: the *Tarsonemus* sp. ( $b = -0.250 \pm 0.125$ ,  $F_{1,78} = 3.97$ ,  $P < 0.05$ ); the pathogen *H. thompsonii* ( $b = -2.38 \pm 1.13$ ,  $F_{1,78} = 4.92$ ,  $P < 0.05$ ); sites ( $F_{3,78} = 5.50$ ,  $P < 0.01$ ); tydeid sp. 2 ( $b = -1.11 \pm 0.35$ ,  $F_{1,78} = 11.05$ ,  $P < 0.01$ ) and minimum temperature ( $b = -0.064 \pm 0.030$ ,  $F_{1,78} = 4.09$ ,  $P < 0.05$ ) were all significant factors, explaining 35% of the variation in density. After accounting for adult numbers, the pathogen was the only significant factor influencing the number of eggs per roll.

#### *Field intrinsic rate of increase*

Monthly lambda (intrinsic rate of increase) for the field sites was  $1.52 \pm \text{SD } 1.51$ . The SD was large because there were only 4 sites and populations were seasonal. This gave the annual field intrinsic rate ( $r$ ) = 5.02 per year, with an estimated doubling time in the field of 50 days.

## **Discussion**

*Floracarus perrepa* has a simple biology without deuterozygotes. This type of biology seems suited to its subtropical/tropical habitat where it feeds and reproduces year round on its host plant. The development times for all stages, oviposition period, and generation time for the females were all significantly shorter at the higher temperature (26°C). However, the net reproductive rate and mean egg production per female were higher at the lower temperature (21°C). These differences indicate that *F. perrepa* is tolerant of the wide range of climatic conditions it experiences during the year. The approximate developmental zero result showed this mite could still continue development at temperatures near 5°C. This result was supported by cold

temperature studies on infested whole plants in which the mites survived for more than two months when held at a constant 7°C (Goolsby, unpublished data). The cold tolerance of *F. perrepae* may allow it to slowly continue development on *L. microphyllum* during the cool winters of its subtropical climate in eastern Australia. In the summer, *F. perrepae* survived temperatures of 30-35°C within the humid microclimate of the roll where it developed independently of the external RH. These conditions differ considerably from those required by *A. chondrilla*, a biological control agent of *Chondrilla juncea* L., which prefers temperatures of 25-28°C during the day, 15-20°C during the night and humidity of 50% for the growth of the galls and the development of colonies (Caresche and Wapshere, 1974).

Leaf rolls were formed by *F. perrepae* in 4.15 days and 3.75 days at 21°C and 26°C, respectively, but the difference was not significant. This duration is similar to that of *Cecidophyes rouhollahi* Craemer, which caused leaf rolling within 3-4 days at 25°C on *Gallium aparine* L. and *Gallium spurium* L. (Craemer *et al.*, 1999). Leaf roll formation is crucial to survival because it provides a protected environment where humidity is constant. The induction of leaf rolls must be rapid and proceed at all temperatures which are characteristic of its local climate. Therefore, the leaf roll response by the plant appears to be one of the most critical interactions between the mite and the plant. Goolsby *et al.* (2004a) reported that *F. perrepae* from southeast Queensland were unable to produce leaf rolls on the invasive Florida genotype of *L. microphyllum*. Different genotypes of *L. microphyllum* may have developed unique plant defenses, which may prohibit leaf roll induction. This process may account for the high level of host specificity in Eriophyidae.

Although the proportion of male *F. perrepae* was low, many spermatophores were observed at the feeding sites. Likewise, *Acalitus phloeocoptes* (Nalepa), which produces galls and deforms fruit spurs on plum and almond (Lindquist and Amrine, 1996), has a field-assessed sex ratio characterized by a predominance of females. The percentage of males in the population was 0.3% for winter, 5% for spring and 1.5% for summer and autumn (Sternlicht *et al.*, 1973). In general, males of *F. perrepae* were consistently present in the population, but in the low numbers seen in other eriophyid species (Sternlicht, 1962; Swirski and Amitai, 1960; Swirski, 1962).

*Floracarus perrepae* was observed to move by both walking and jumping. The jumping behaviour observed is a very useful dispersal mechanism. Jumping

allows the mite to become airborne and would assist passive dispersal of the mite via wind currents over long distances in the field. This behaviour has been previously observed by Smith (1960), Nault and Styer (1969) and Ozman and Toros (1997) in eriophyid mites. Passive dispersal between plant stands could be a useful life trait if this mite is to be used as a biological control agent in the Florida Everglades, as stands of *L. microphyllum* are patchy and disjunct, often being separated by vast areas of saw-grass marshes.

Several factors in the biological studies, including generation time, intrinsic rate of increase, and population doubling time indicated that *F. perrepae* is capable of rapid population increases. This ability to rapidly build its population should enable the mite to exploit favourable climatic conditions and potential lack of specialist natural enemies in Florida.

In the field studies, several factors were found to play significant roles in the phenology of *F. perrepae*. Populations of the mite, as measured by the number of leaf rolls (plant damage) increased following periods of high soil moisture. The proportional increase in plant damage caused by the mite could be due to lower levels of mortality experienced by dispersing females during periods of high humidity and soil moisture. During dispersal and the search for suitable new growth, females must spend several days feeding on the outside of the subpinnae before a new, protective leaf roll can be produced. Unfavourable conditions such as hot, dry weather could cause high levels of mortality to the dispersing females. Weather conditions that produce high levels of soil moisture help create environmental conditions favourable for dispersal and colonization. Average soil moisture and humidity levels in Florida are very similar to southeast Queensland and conditions for *F. perrepae* are therefore assumed to be favourable. The climate-matching program CLIMEX (Sutherst *et al.*, 1999) calculates an ecoclimatic index of similarity of 79 for Tewantin, Queensland (near Bribie Island), and West Palm Beach, Florida. An index value of 100 indicates an identical climate; so southeast Queensland and south Florida have very similar climates. Therefore, it seems likely that abiotic factors which positively influence populations of *F. perrepae* in Queensland will be similar in Florida.

Inside the leaf roll, a different set of factors influenced *F. perrepae* adults, nymphs, larvae and eggs. Conditions inside the roll are presumed to be near 100% RH, since the mites are almost totally enveloped in plant tissue. Increasing temperatures and natural enemies had significant impacts on the mite stages within the leaf roll. As

temperatures rose, all stages of the mite were negatively affected. This is consistent with the biological studies which showed a decrease in fecundity, development time and survival longevity from 21°C to 26°C.

Natural enemies also had a significant impact, especially tydeid sp. 2. In 2002, populations of predatory mites were higher than in 2001. This increase correlated with lower numbers of *F. perrepae* in the leaf rolls (Figures 3B&C). *Tarsonemus* sp. was not as common in 2002 as in 2001, thus most predation was assumed to have been caused by the tydeid sp. 2. Despite the higher level of predation, *F. perrepae* populations still caused consistent damage to *L. microphyllum*. Even if co-adapted predatory mites occur in Florida it is predicted that the impact of these predators would not be greater than that determined in Australia. Therefore, it is reasonable to predict that the population levels of *F. perrepae* in Florida would be similar to those documented in Australia. Population levels similar to those reported by Goolsby *et al.* (2004b) in field impact studies, should cause significant reductions in biomass production by *L. microphyllum* in Florida.

Pratt *et al.* (2002) calculated the yearly intrinsic rate of increase for *Oxyops vitiosa* Pascoe (Coleoptera: Curculionidae) at 2.44 per year. They compared the rate for *O. vitiosa* with two other well known biological control agents, *Cactoblastis cactorum* Bergroth (Lepidoptera: Pyralidae) and *Galerucella californiensis* (L.) (Coleoptera: Curculionidae), that had previously been calculated at 3.38 and 2.24, respectively. Our estimates for *F. perrepae* are slightly higher at  $r = 5.02$ . The higher rate of increase reflects the shorter generation time of *F. perrepae* and its high fecundity. Although this figure is an estimate based on only one year of field data from the native range, it does show the potential for this mite to rapidly reach high population densities. Rates of increase could be even higher in Florida if the impact of predators and pathogen is less than that experienced in the native range.

Considering the results of these studies, *F. perrepae* has many of the characteristics of an effective biological control agent. It causes considerable damage to the plant by inducing leaf roll galls, has a short generation time, and is capable of rapidly increasing its population when climatic conditions are favourable. The genotype of the *F. perrepae* evaluated in this study is unique and other genotypes are known to exist throughout the native range (Goolsby *et al.*, 2004a). Further studies are needed to select the best-adapted genotype for the invasive Florida form of *L. microphyllum*. Other eriophyid mites have been found to be highly specialized, even specific to certain races or forms of their host (Sobhian and Andres 1978; Cullen and

Moore, 1983). The developmental parameters and phenology of other genotypes of *F. perrepae* could be different to those described in this study. Consideration of genotypic variation in *F. perrepae* and its host *L. microphyllum*, along with the evolutionary constraints of its environmental tolerance, are critical to the understanding of this highly co-adapted herbivore.

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**Table 1. Life history parameters of *Floracarus perrepae* reared on *Lygodium microphyllum* at two temperatures\*.**

Parameters	21°C		26°C	
	(Days) ± SE	n	(Days) ± SE	n
Egg	5.03 ± 0.10 a	35	3.92 ± 0.17 b	18
Larva	0.96 ± 0.03 a	35	0.81 ± 0.07 b	18
Nymphochrysalis	1.07 ± 0.05 a	35	0.81 ± 0.07 b	18
Nymph	0.83 ± 0.07 a	35	0.72 ± 0.06 b	18
Imagochrysalis	1.06 ± 0.05 a	35	0.78 ± 0.06 b	18
Larva to adult	3.91 ± 0.10 a	35	3.11 ± 0.09 b	18
Total immature period	8.94 ± 0.09 a	35	7.03 ± 0.14 b	18
Preoviposition period	2.83 ± 0.21 a	12	1.77 ± 0.20 b	13
Oviposition period	26.17 ± 1.55 a	12	16.46 ± 1.04 b	13
Post oviposition period	1.58 ± 0.23 a	12	1.15 ± 0.34 a	13
Adult longevity	30.58 ± 1.55 a	12	19.38 ± 1.23 b	13

\*Means in rows followed by the same letter are not significantly different ( $P < 0.05$ ).

**Table 2. Life table parameters of *Floracarus perrepae* reared on *Lygodium microphyllum* at two temperatures.**

<b>Parameters</b>	<b>21°C</b>	<b>26°C</b>
Proportional immature mortality	0.054	0.117
Net reproductive rate	47.20 female eggs/female	31.04 female eggs/female
Intrinsic rate of increase	0.186/day	0.242/day
Generation time	20.7 days	14.2 days
Population doubling time	3.73 days	2.86 days

**Table 3. Mean numbers of adult and immature *Floracarus perrepae*, the proportion of leaf rolls and mean densities at four field sites in southeast Queensland in a two-year study\*.**

<b>Field sites</b>	<b>Mean no.s of mites per leaf roll <math>\pm</math> SE</b>	<b>Proportion of leaf rolls <math>\pm</math> SE</b>	<b>Mean densities of mites <math>\pm</math> SE**</b>
Lagoon Road	7.82 $\pm$ 1.47a	0.291 $\pm$ 0.033a	2.37 $\pm$ 0.55a
Carbrook Creek	12.11 $\pm$ 1.56b	0.365 $\pm$ 0.022ab	4.62 $\pm$ 0.70b
Gallagher's Point	7.11 $\pm$ 1.08a	0.324 $\pm$ 0.031a	2.25 $\pm$ 0.35a
McMahon Road	7.13 $\pm$ 1.65a	0.433 $\pm$ 0.053b	3.18 $\pm$ 0.67ab

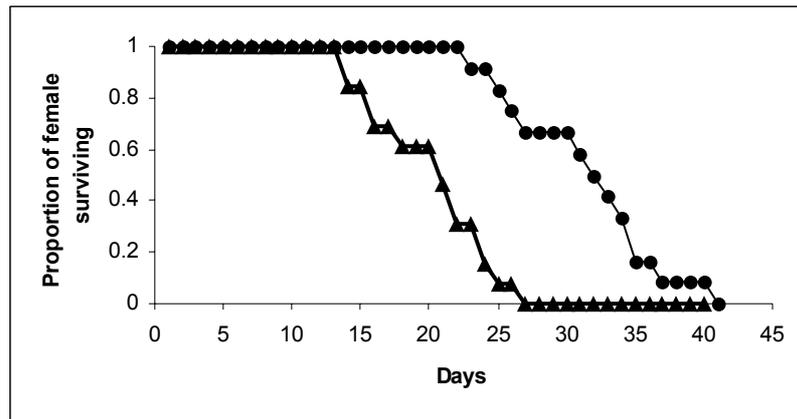
\*Means in columns followed by the same letter are not significantly different (P<0.05).

\*\*Density was calculated as the product of mean number of mites per leaf roll and the proportion of leaf rolls.

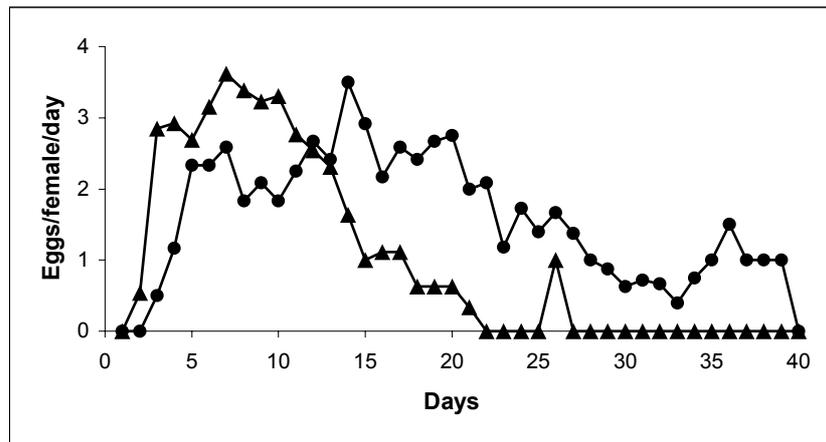
**Table 4. Mean numbers of *Floracarus perrepae* life stages per roll at four sites in southeast Queensland\*.**

<b>Field sites</b>	<b>Adults</b>	<b>Immatures</b>	<b>Eggs</b>
Lagoon Road	3.85 ± 0.77a	3.97 ± 0.79a	7.53 ± 1.15a
Carbrook Creek	5.27 ± 0.56b	7.30 ± 1.18b	14.63 ± 1.79b
Gallaghers Point	3.29 ± 0.43a	4.06 ± 0.73a	8.71 ± 1.39a
McMahon Road	3.29 ± 0.77a	3.84 ± 0.94a	6.44 ± 1.50a

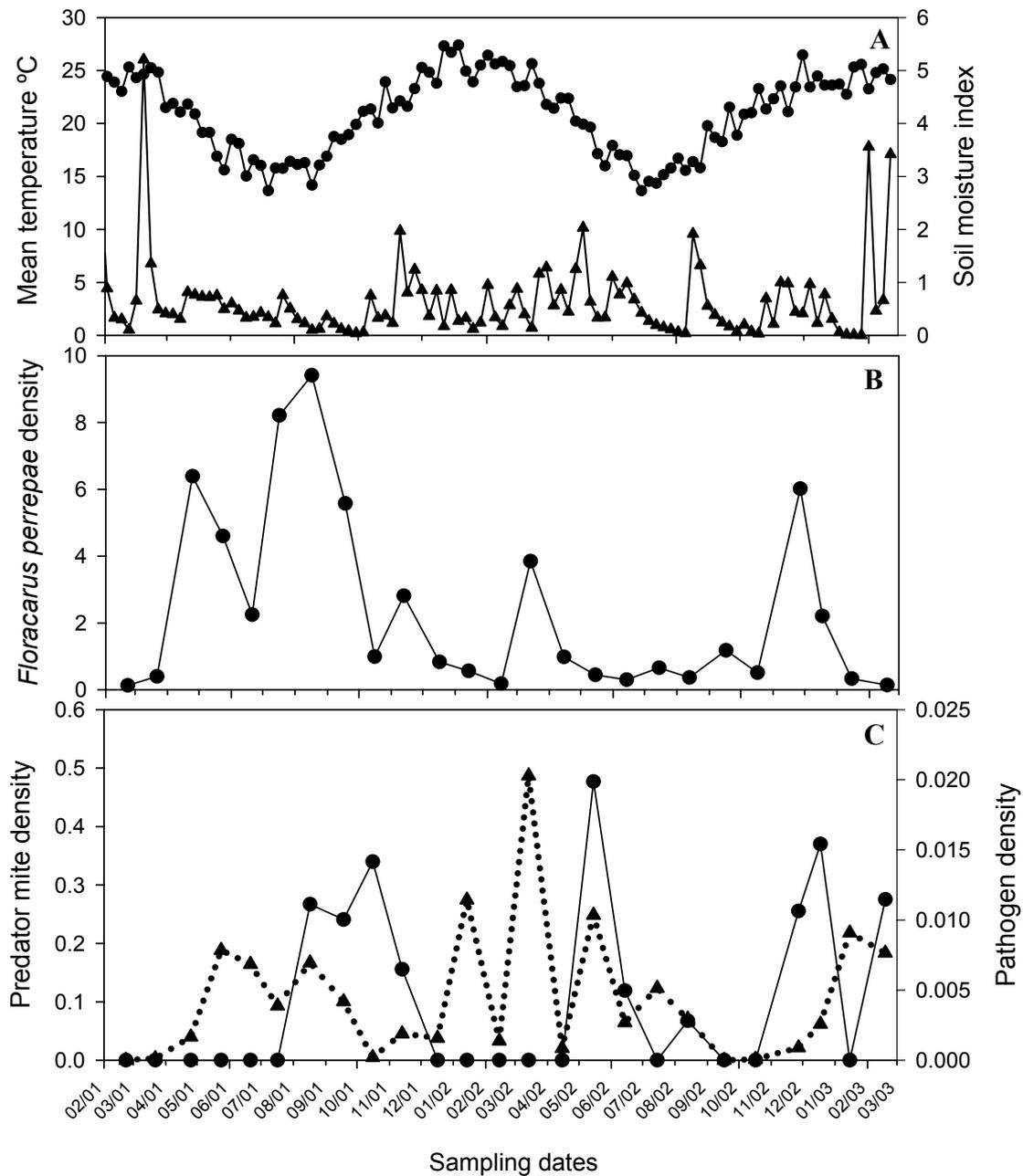
\*Means in columns followed by the same letter are not statistically different when analyzed on the square root scale ( $P < 0.05$ ).



**Figure 1.** Proportional survival of *Floracarus perrepa* females at two temperatures. Lines with (●) and (▲) represent 21°C and 26°C, respectively.



**Figure 2.** Mean egg lay per day by *F. perrepae* females. Lines with (●) and (▲) represent 21°C and 26°C, respectively.



**Figure 3.** Biotic and abiotic factors influencing seasonal phenology of *Floracarus perrepae* over a two-year period at Logan Road, southeast Queensland. A) Weekly mean temperature (●) and soil moisture index(▲), with a value of 1 indicating soil saturation and runoff; B) Mean monthly density of *F. perrepae*, calculated as the product of mites per leaf roll and proportion of leaf rolls; C) Mean monthly densities of predatory mites per leaf roll (...▲...) and density of *Hirsutella thompsonii* (—●—) calculated as the product of the proportion of leaf rolls with infection and the proportion of leaf rolls.