

# Utilization of aphid honeydew and floral nectar by *Pseudacteon tricuspis* (Diptera: Phoridae), a parasitoid of imported fire ants, *Solenopsis* spp. (Hymenoptera: Formicidae)

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## Abstract

We examined the effects of cotton aphid honeydew and buckwheat floral nectar on the lifespan and body carbohydrate nutrient levels of the phorid fly, *Pseudacteon tricuspis* (Diptera: Phoridae). Compared to flies provided with water only, cotton aphid honeydew modestly but significantly increased longevity of female and male *P. tricuspis* by about 1 day, with significant increases in the body levels of fructose, total sugars, and glycogen. Age had a positive effect on the levels of total sugars and glycogen in honeydew-fed flies. However, sex and mating had no significant effect on longevity and body nutrient levels. Body size (as measured by wing length) was positively correlated with glycogen levels in males, with larger males accumulating more glycogen from honeydew than smaller males. *P. tricuspis* flies were observed foraging on buckwheat flowers. However, no significant effects of buckwheat nectar on phorid fly longevity and body nutrient levels were detected. These results may suggest that buckwheat florets are morphologically incompatible with the mouthparts of phorid flies, or the possibility of innate behavioral characteristics of phorid flies that limit nectivory. Alternatively, buckwheat nectar may be quantitatively or qualitatively inferior to cotton aphid honeydew or may contain compounds that are mildly repellent to *P. tricuspis*. Future studies will investigate the mechanisms behind the observed reduced suitability of buckwheat nectar as a food source for *P. tricuspis*. Conservation of naturally occurring sugar sources in the field, including exposed sugar sources such as honeydew, may promote *P. tricuspis* lifespan and possibly, its impact as a biological control agent against imported fire ants, *Solenopsis* spp. (Hymenoptera: Formicidae).

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**Keywords:** *Pseudacteon tricuspis*; *Solenopsis invicta*; Longevity; Nectar; Honeydew; Fructose; Total sugars; Glycogen; Parasitoid; Habitat manipulation; Biological control

## 1. Introduction

Many parasitoids require carbohydrate foods as an energy source during their adult stage. In the field, sugar is naturally available to parasitoids primarily in the form of nectar (either floral or extrafloral) and homopteran honeydew (Bugg et al., 1989; Evans, 1993; Jervis et al., 1993, 1996; Rogers, 1985). Numerous laboratory and field studies have reported significant effects of sugar

feeding on longevity and/or fecundity parasitoids (Dyer and Landis, 1996; England and Evans, 1997; Fadamiro and Heimpel, 2001; Foster and Ruesink, 1984; Hagley and Barber, 1992; Heimpel et al., 1997; Idris and Grafius, 1995; Jacob and Evans, 2000; Lee et al., 2004; Olson and Andow, 1998; Olson et al., 2000). Some of these studies also showed significant increases in body nutrient (mainly carbohydrate nutrients) levels of sugar-fed wasps (Fadamiro and Heimpel, 2001; Lee et al., 2004; Olson et al., 2000).

Sugar feeding may have a positive effect on parasitoid reproduction directly or indirectly. Parasitoids with

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synovigenic life history may benefit directly from sugar feeding by maturing additional eggs (Binns, 1980; Heimpel and Jervis, 2005; Jervis et al., 2001; Olson and Andow, 1998). Pollen, which is often ingested with nectar may also enhance reproduction in many species by providing nutrient resources for egg production (Jervis et al., 1996). In addition, Heimpel et al. (1997) postulated that sugar feeding may prevent egg resorption by female parasitoids. The increased lifespan that usually results from feeding on sugar may indirectly enhance parasitoid reproduction by allowing more time for location and attack of more hosts (Heimpel and Jervis, 2005; Idris and Grafius, 1995). Based on the demonstrated impact of sugar feeding on the lifespan and reproduction of several parasitoid species, and the assumption that suitable floral sources are limiting in many agroecosystems, provision of artificial sugar supplements or planting of nectar-producing flowering plants is now commonly recommended and utilized in biological control programs (Jacob and Evans, 1998). However, naturally occurring homopteran honeydew may still be a key sugar source for foraging parasitoids in agroecosystems, and its potential utilization by different species of parasitoids needs to be explored further.

Qualitative and quantitative differences are known to exist in the composition of nectar and honeydew sugars (Baker and Baker, 1983; van Handel et al., 1972; Wäckers, 2001). Nectar and honeydew sugars contain the same primary components, the disaccharide sucrose and its two monosaccharide components, glucose and fructose (Baker and Baker, 1983; van Handel et al., 1972; Wäckers, 2001). However, honeydew also contains several disaccharides, such as maltose (Völkl et al., 1999) and melibiose, and trisaccharides, such as melezitose, raffinose, and erlose (Baker and Baker, 1983; Wäckers, 2001). These disaccharides and trisaccharides are rare or missing in nectar, act as honeydew-specific signature sugars, and may potentially affect the suitability and utilization of honeydew by parasitoid species. For instance, Wäckers (1999, 2001) reported a higher feeding response by *Cotesia glomerata* (L.) (Hymenoptera: Braconidae) to monosaccharide sugars compared to trisaccharides, suggesting reduced suitability of honeydew sugars. In addition, melezitose and raffinose, components of honeydew sugars are known to form crystals within a short time, hence reducing their availability to parasitoids (Chen and Fadamiro, unpublished data; Wäckers, 2001). In contrast, Beach et al. (2003) found that several honeydew sugars were readily accepted by the egg parasitoid, *Anaphes iole* Girault (Hymenoptera: Mymaridae), suggesting that different species may have different sugar preferences.

Despite the relatively large volume of literature on sugar feeding by hymenopteran parasitoids, little is known on sugar utilization by non-hymenopteran parasitoids. Recently, we demonstrated sugar feeding by a

dipteran parasitoid, the phorid fly, *Pseudacteon tricuspis* Borgmeier (Diptera: Phoridae) (Chen et al., 2005; Fadamiro et al., 2005). Because of their high host specificity and sufficient impact on imported fire ants, *Solenopsis* spp. (Hymenoptera: Formicidae), *Pseudacteon* phorid flies are considered promising biocontrol agents of imported fire ants in the United States (Feener and Brown, 1992; Gilbert, 1996; Porter, 1998). Since the last decade, two species of phorid flies, *P. tricuspis* and *Pseudacteon curvatus*, have been continuously released in many parts of southern United States (Graham et al., 2003; Porter et al., 1999, 2004), and attempts are currently being made to monitor parasitoid establishment and impact (Morrison and Porter, 2005). Although several aspects of the behavior and biology of *Pseudacteon* phorid flies have been investigated (Morrison, 2000; Porter, 1998), little is known about the nutritional ecology and foraging behavior of phorid flies in nature. Adult phorids are presumed to be generalist feeders on plant nectar, sap, or honeydew (Morrison, 2000), and there are a few casual observations of water drinking or sugar feeding by some fly species in the family Phoridae (Disney, 1994; Pesquero et al., 1995; Porter et al., 1997). Apart from these few anecdotal reports, we are not aware of any previously conducted systematic studies to demonstrate potential sugar feeding by phorid flies in the field.

Having demonstrated in earlier studies that adult *P. tricuspis* are capable of feeding on artificial sugar sources (sucrose solution), and that sugar feeding resulted in significant increases in longevity and accumulation of body carbohydrate nutrients (Chen et al., 2005; Fadamiro et al., 2005), we became interested in investigating potential utilization of naturally occurring sugar sources by phorid flies in the field. In the current study, we examined the suitability of aphid honeydew and floral nectar as food sources for *P. tricuspis*, and compared the effects of both sugar sources on the lifespan and nutrient accumulation in adult flies.

## 2. Materials and methods

### 2.1. Food sources

We compared the suitability of nectar from buckwheat flowers, *Fagopyrum esculentum* Moench and honeydew produced by cotton aphid, *Aphis gossypii* Glover (Homoptera: Aphididae) as sugar sources for *P. tricuspis*. Buckwheat was selected primarily because of its accessible nectar, and also because it is commonly utilized in similar studies and in habitat manipulation programs (Lee et al., 2004; Nicholls et al., 2000; Stephens et al., 1998). We tested cotton aphid honeydew because of its prevalence in agroecosystems in the southern United States where cotton production remains

widespread. Due to its widespread abundance and the proximity of phorid fly release sites to cotton fields, cotton aphid honeydew is perhaps the most likely honeydew to be encountered by foraging phorid flies released for biological control of imported fire ants in southern United States. Cotton aphid honeydew was obtained by rearing field collected cotton aphids on cotton plants, *Gossypium hirsutum* L. Cotton plants were grown on Pro-Mix medium in 20.3-cm diameter plastic pots in a greenhouse at about  $27 \pm 1^\circ\text{C}$ ,  $\sim 70\%$  r.h., and a photoperiod of L16:D8. The cotton variety utilized was Sure-Grow 747, a 'Round-Up Ready' conventional cultivar with minimal production of extrafloral nectar plants. Buckwheat plants were also grown in the greenhouse under similar conditions. All plants were grown using standard greenhouse practices and were watered every three days and fertilized every two weeks. Fresh buckwheat flowers were maintained throughout the test period by sowing buckwheat twice every week.

## 2.2. Parasitoids

Adult *P. tricuspis* used in this study were supplied by the fire ant rearing facility of the USDA-ARS, Center for Medical, Agricultural and Veterinary Entomology, Gainesville, FL, USA. The phorid flies were reared at this facility on workers of red imported fire ant, *Solenopsis invicta* Buren using the methods described by Porter et al. (1997), and shipped to us as parasitized fire ant worker heads. Parasitized fire ant worker heads were received in batches and kept in a plastic jar ( $25 \times 13$  cm) with a lid until emergence. Emerging flies did not have access to food or water in the jar. Newly emerged flies were removed promptly with an aspirator and sexed immediately under a dissecting microscope using presence or absence of the distinct female ovipositor.

## 2.3. Parasitoid longevity

The survivorship of adult female and male *P. tricuspis* was compared across four feeding treatments: (1) water only (starved), (2) a bundle of buckwheat flowers with nectar, (3) a cotton leaf infested with cotton aphids producing honeydew, and (4) a clean (uninfested) fresh cotton leaf. The uninfested cotton leaf constituted an additional control treatment to examine if phorid flies could obtain extrafloral nutrients from cotton leaves. Extrafloral nectaries are known to occur on the leaves of several plants (Wäckers, 2002), including various cotton cultivars such as the one utilized in this study. Water was provided in all treatments by filling a 0.5 ml microcentrifuge tube with distilled water and threading a cotton string through a hole in the cap of the tube, and water tubes were refilled as needed. Flowers used in the buckwheat nectar treatment were inserted in water-filled tubes. We also tested the effect of mating (mated vs.

unmated) on survivorship of female and male *P. tricuspis*. The various combinations of food and mating status resulted in a total of 8 treatment combinations for each sex. Newly emerged flies were placed in groups of two individuals either of the same sex (unmated treatments) or of opposite sex (mated treatments) in a 15-cm diameter plastic Petri dish with the presence of different food sources. Petri dishes were kept in a growth chamber at  $25 \pm 1^\circ\text{C}$ ,  $75 \pm 5\%$  r.h., and a photoperiod of L14:D10. Saturated brine was used to maintain constant relative humidity in the chamber. Female and male *P. tricuspis* emerging on the same day were distributed evenly across the treatment combinations. At least 12 flies of each sex were tested for each diet and mating combination. Petri dishes were checked once daily for survival and dead flies were promptly removed. To ensure availability of fresh sugar sources, flowers were replaced in the Petri dishes every other day, while cotton leaves were replaced every day. Effects of diet, sex, and mating status on survivorship were evaluated using proportional hazard modeling (SAS Institute, 1998). This model also allowed testing for any significant two-way or three-way interactions among the three variables. Survivorship data for each sex were analyzed separately by using analysis of variance (ANOVA) followed by the Tukey–Kramer HSD test for multiple comparisons of means at  $P < 0.05$  (SAS Institute, 1998).

## 2.4. Body nutrient analyses

Daily changes in the amounts of fructose, total sugars, and glycogen were quantified for phorid flies placed in each of the first three treatments described in the longevity experiment: water only (starved); buckwheat flowers with nectar; and a cotton leaf infested with cotton aphids producing honeydew. The test protocols were similar to those described by Fadamiro et al. (2005). Female and male *P. tricuspis* emerging on the same day were placed in groups of two individuals of the same sex within 15-cm diameter plastic Petri dishes, and randomly assigned evenly to the three treatments. Live flies from each treatment were collected, frozen at  $-20^\circ\text{C}$ , and assayed daily from ages 1 to 4 days in the honeydew and nectar treatments. Because sugar-starved flies rarely lived beyond 3 days under our test conditions, nutrient assays were conducted only for 1- to 3-day-old flies in the water only treatment. Forewing length was used to estimate fly size. One forewing was pulled from each frozen fly, slide mounted and measured to the nearest 0.05 mm on a dissecting microscope. The amount of each nutrient obtained from newly emerged unfed (day 0) flies was regarded as the general amount present in female and male flies at emergence. At least 10 females and 8 males were bioassayed daily for each diet treatment.

The amounts of fructose, total sugars, and glycogen in individual flies were quantified using a series of

standard biochemical tests originally developed for mosquitoes (van Handel, 1965), modified for parasitoid wasps (Fadamiro and Heimpel, 2001; Lee et al., 2004; Olson et al., 2000), and recently adapted for phorid flies (Fadamiro et al., 2005). The methodology and protocols used in the current study to quantify body nutrient levels in phorid flies have been described in detail by Fadamiro et al. (2005). Lipid levels of flies used in this study were not analyzed since adult *P. tricuspsis* are not capable of converting dietary sucrose to lipids (Fadamiro et al., 2005).

Nutrient data were analyzed by using multiple regression analysis to test the effects of diet, age, diet  $\times$  age interaction, and wing length on nutrient levels. In the first analysis, we compared nutrient levels among the three diet treatments. In the second analysis, we compared nutrient levels between nectar-fed and honeydew-fed flies only. Each sex was analyzed separately since male *P. tricuspsis* are smaller and with lower nutrient contents than females (Fadamiro et al., 2005). Nutrient data were checked for unequal variances, and if necessary transformed using log transformation (SAS Institute, 1998) to equalize variances. Data were further analyzed by using analysis of variance and Tukey HSD for multiple comparisons (SAS Institute, 1998), comparing nutrient levels among flies of the same sex and age provided different diet treatments. Mean nutrient levels were compared among the three diet treatments on days 1–3, and only between nectar-fed and honeydew-fed flies on day 4 since flies provided water only rarely lived beyond 3 days. Statistical analyses were run on absolute nutrient amounts rather than on absorbance values. Newly emerged flies were not included in the statistical analyses since they were not part of any of the diet treatments.

### 3. Results

#### 3.1. Parasitoid longevity

Diet had a significant and positive effect on lifespan of adult *P. tricuspsis* ( $\chi^2=15.6$ ,  $df=3$ ,  $P=0.001$ ). However, mating ( $\chi^2=0.06$ ,  $df=1$ ,  $P=0.81$ ) and sex ( $\chi^2=0.08$ ,  $df=1$ ,  $P=0.78$ ) did not significantly affect longevity. Similarly, no significant interactions were recorded between diet and sex ( $\chi^2=1.8$ ,  $df=3$ ,  $P=0.62$ ), diet and mating ( $\chi^2=0.3$ ,  $df=3$ ,  $P=0.96$ ), sex and mating ( $\chi^2=0.2$ ,  $df=1$ ,  $P=0.68$ ), or diet, sex, and mating ( $\chi^2=1.1$ ,  $df=3$ ,  $P=0.77$ ). Since mating had no significant effect on longevity, data from unmated and mated flies were pooled and then reanalyzed by using ANOVA to test for the effect of diet on longevity of female and male flies provided the four diet treatments. The results also showed a significant effect of diet on longevity of female ( $F=7.3$ ,  $df=3$ ,  $P=0.0002$ ) and male ( $F=2.9$ ,  $df=3$ ,  $P=0.04$ ) *P. tricuspsis*.

Table 1

Mean longevity (days  $\pm$  SE) of female and male *P. tricuspsis* provided different diet treatments

Diet	Females	Males
Water only	3.4 $\pm$ 0.2 bc	3.2 $\pm$ 0.2 b
Buckwheat nectar	3.9 $\pm$ 0.1 ab	3.7 $\pm$ 0.3 ab
Cotton aphid honeydew	4.3 $\pm$ 0.2 a	4.2 $\pm$ 0.3 a
Clean cotton leaf	3.2 $\pm$ 0.2 c	3.5 $\pm$ 0.2 ab
<i>F</i>	7.3	2.9
<i>P</i>	0.0002	0.03

Means within the same column having different letters are significant (Tukey–Kramer HSD,  $P < 0.05$ ).

When maintained continuously with open flowers or cotton leaves containing honeydew, adult *P. tricuspsis* flies were observed foraging on the inflorescences or on the surface of honeydew-laden cotton leaves. Female and male *P. tricuspsis* fed cotton aphid honeydew had a modestly but significantly greater longevity than flies provided water only; honeydew increased longevity by about 1 day compared to flies provided water only (Table 1). However, provision of buckwheat nectar did not increase phorid fly longevity. Similarly, flies provided clean (uninfested) cotton leaf had similar longevity as water-fed flies, suggesting that the potential availability of extrafloral nectar on the leaves of the cotton cultivar utilized in this experiment was not sufficient to enhance longevity of adult *P. tricuspsis* (Table 1). Survivorship curves showing longevity of flies fed the different diet treatments are shown in Fig. 1. The greatest survivorship was recorded for flies fed cotton aphid honeydew with some individual males surviving for up to 7 days, compared to the maximum longevity of 4 and 5 days recorded for males in the water only and nectar treatments, respectively (Fig. 1).

#### 3.2. Body nutrient analyses

Multiple regression analyses revealed a significant effect of diet on nutrient levels of female and male *P. tricuspsis* fed the three diet treatments (Table 2). There was also a significant effect of age on total sugars and glycogen levels of female and male flies, with levels generally decreasing with age in sugar-starved flies while increasing with age or remaining stable in honeydew-fed flies. In general, body size as measured by wing length had no significant effect on nutrient levels, with the exception of glycogen levels in males, with larger males accumulating more glycogen from honeydew than smaller males. Also, no significant diet  $\times$  age interactions were recorded on nutrient levels (Table 2). Similar results were obtained when only buckwheat nectar and cotton aphid honeydew were compared in the analysis (Table 2).

*Pseudacteon tricuspsis* emerged with less than 1  $\mu$ g ( $\sim 0.58$   $\mu$ g) of fructose as measured by the cold anthrone

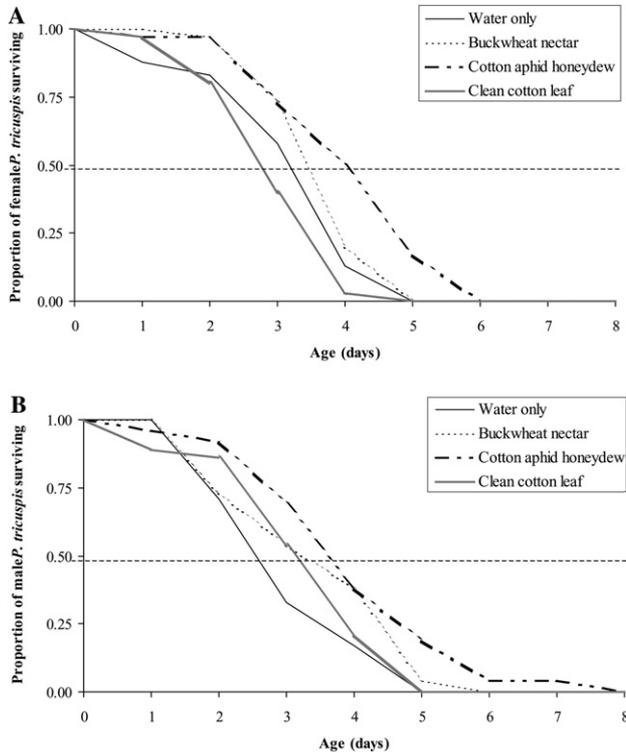


Fig. 1. Survivorship curves for female (A) and male (B) *P. tricuspidis* provided water only, buckwheat nectar, cotton aphid honeydew, or clean (uninfested) cotton leaf. Dashed line at 0.5 survivorship indicates median longevity for each treatment.

test in this study. Therefore, fructose levels greater than  $1\ \mu\text{g}$  may indicate sugar feeding by *P. tricuspidis* (Fadamiro et al., 2005). Expectedly, fructose levels in flies provided water only did not increase beyond  $1\ \mu\text{g}$  throughout their lifespan (Fig. 2). Fructose levels of females fed buckwheat nectar averaged  $>2\ \mu\text{g}$  on day 1 but declined rapidly to  $<1\ \mu\text{g}$  on day 2, remaining at this very low level throughout their lifespan. This may indicate some degree of sugar feeding by 1-day-old nectar-fed female flies. Fructose levels in nectar-fed male flies were consistently lower than  $1\ \mu\text{g}$ , suggesting the absence of sugar feeding. In contrast, fructose levels detected in female and male flies fed cotton aphid honeydew were consistently greater than  $1\ \mu\text{g}$  throughout their lifespan, indicating sugar feeding (Fig. 2). Significantly greater fructose amounts were detected in honeydew-fed females on day 2, compared to nectar-fed females, or to females provided water only. For males, slightly higher fructose levels were generally recorded for honeydew-fed flies, but fructose levels in honeydew-fed males were significantly greater than levels in nectar-fed males only on day 4 (Fig. 2).

Adult *P. tricuspidis* emerged with  $\sim 5\ \mu\text{g}$  of total sugars (Fig. 3). This moderate teneral level of total sugars was maintained for one day in flies provided water only, but declined gradually thereafter. The emergent level of total sugars ( $5\ \mu\text{g}$ ) was maintained in nectar-fed flies through-

out their lifespan with no significant increases in total sugar levels (Fig. 3). However, female *P. tricuspidis* fed honeydew significantly increased their total sugar levels on the first day of feeding by  $\sim 10\ \mu\text{g}$  and maintained this relatively high level of total sugars throughout their lifespan. Total sugar levels in honeydew-fed females were significantly greater than levels in nectar-fed females on days 1, 2, and 4, and greater than levels in females provided water only on days 1 and 2. For honeydew-fed males,  $\sim 10\ \mu\text{g}$  of total sugars was detected on the first day of feeding, increasing to  $\sim 20\ \mu\text{g}$  on day 3. Significantly greater levels of total sugars were detected in honeydew-fed males on days 2 and 4, compared to males in the nectar treatment, and on days 2 and 3, compared to males provided water only. Endpoint (day 4) total sugar levels were significantly greater in honeydew-fed female and male flies than in their nectar-fed counterparts (Fig. 3).

Emergent level of glycogen in female and male *P. tricuspidis* used in this study was about  $4\ \mu\text{g}$  (Fig. 4). This low teneral glycogen level was maintained over the lifetime of flies provided water only and in nectar-fed flies (Fig. 4). Significant increases in glycogen levels were, however, recorded for female and male flies fed honeydew (Fig. 4). One day of honeydew feeding resulted in a significant increase in glycogen level ( $\sim 6\ \mu\text{g}$ ) in male flies, whereas only a slight increase in glycogen level was recorded in honeydew-fed females on day 1. Glycogen levels detected in honeydew-fed female flies were significantly greater than levels in females provided water only on day 3, and greater than levels in nectar-fed females on day 4 (Fig. 4). Also, glycogen levels on days 2 and 3 were greater in honeydew-fed males than in males provided water only, and greater on days 3 and 4 in honeydew-fed males than in nectar-fed males (Fig. 4). Endpoint glycogen levels in honeydew-fed flies were about two times higher than in nectar-fed flies.

#### 4. Discussion

The results of this study showed that adult *P. tricuspidis* were capable of feeding on cotton aphid honeydew with significant increases in longevity and body carbohydrate nutrient levels. Adult phorid flies were frequently observed foraging on cotton leaves with aphid honeydew, but rarely observed foraging on honeydew-free cotton leaves. However, the longevity and nutrient levels of flies fed buckwheat nectar were not significantly different from those of flies provided water only. Previous studies on the relative effect of floral nectar and homopteran honeydew on parasitoid longevity have produced conflicting results. Several authors have reported a greater effect of honeydew on parasitoid longevity compared to floral nectar (Foster and Ruesink, 1984; Hagley and Barber, 1992). For instance, adult *Bathyplectes curculionis*

Table 2

Multiple regression analyses testing for effects of diet, age, the interaction between diet and age, and wing length on nutrient levels of female and male *P. tricuspis*

	df	Females			Males		
		MS	F	P	MS	F	P
<i>Fructose (3 diet treatments: water only vs. nectar vs. honeydew)</i>							
Diet	2	12	9.0	0.0003	6.3	4.9	0.01
Age	1	0.4	0.31	0.58	0.01	0.01	0.96
Diet × Age	2	0.01	0.003	0.99	2.4	1.9	0.16
Wing length	1	0.02	0.02	0.89	0.03	0.02	0.88
<i>Fructose (nectar vs. honeydew)</i>							
Diet	1	17.9	14.5	0.0003	9.7	8.9	0.005
Age	1	0.6	0.47	0.49	0.6	0.55	0.46
Diet × Age	1	0.01	0.01	0.99	4.1	3.8	0.06
Wing length	1	0.11	0.09	0.76	0.4	0.41	0.53
<i>Total sugars (3 diet treatments: water only vs. nectar vs. honeydew)</i>							
Diet	2	16.5	18.6	<0.0001	12.7	25.3	<0.0001
Age	1	5.5	6.2	0.02	3.0	6.0	0.02
Diet × Age	2	1.3	1.5	0.23	3.8	7.4	0.001
Wing length	1	0.8	0.85	0.36	1.7	3.3	0.07
<i>Total sugars (nectar vs. honeydew)</i>							
Diet	1	23.7	25.5	<0.0001	15.2	28.3	<0.0001
Age	1	1.1	1.2	0.28	0.04	0.08	0.77
Diet × Age	1	0.05	0.06	0.81	1.9	3.6	0.06
Wing length	1	0.3	0.28	0.60	0.08	0.15	0.70
<i>Glycogen (3 diet treatments: water only vs. nectar vs. honeydew)</i>							
Diet	2	4.7	8.5	0.0004	6.8	17.2	<0.0001
Age	1	5.0	9.1	0.003	1.5	3.9	0.05
Diet × Age	2	1.5	2.8	0.07	0.3	0.6	0.55
Wing length	1	1.2	2.2	0.14	3.4	8.6	0.004
<i>Glycogen (nectar vs. honeydew)</i>							
Diet	1	8.1	17.5	<0.0001	12.4	31.1	<0.0001
Age	1	2.9	6.3	0.01	0.54	1.4	0.25
Diet × Age	1	2.6	5.6	0.02	0.08	0.20	0.65
Wing length	1	0.02	0.06	0.80	2.2	5.4	0.02

In the first analysis, three diet treatments (water vs. buckwheat nectar vs. cotton aphid honeydew) were compared. In the second analysis, only buckwheat nectar and cotton aphid honeydew were compared.

(Thompson) (Hymenoptera: Ichneumonidae) reportedly had a 50% increase in longevity when fed aphid honeydew or sugar solution (England and Evans, 1997), compared to the slight (about 14%) increase in longevity recorded for wasps that had access to dandelion flowers in another study (Jacob and Evans, 2000). Wäckers and Steppuhn (2003) reported that the majority (80%) of *Cotesia glomerata* (L.) collected from fields with abundant floral nectar had fed on honeydew, while only 11% fed solely on nectar, indicating significant utilization of honeydew even in the presence of nectar. In contrast, many studies have reported the superiority of floral nectar over homopteran honeydew as sugar sources for several species of hymenopteran parasitoids (Avidov et al., 1970; Lee et al., 2004; McDougall and Mills, 1997).

Several insect- and plant-related factors may account for the lack of utilization of buckwheat nectar by *P. tricuspis* recorded in the current study. Flowering plant species vary considerably in floral morphology, flower attractiveness, and nectar accessibility with the

potential for variable effects on parasitoid foraging and lifespan (Jervis et al., 1996; Patt et al., 1997; Wäckers, 2004). Wäckers (2004) evaluated the suitability of 11 flowering plants as food sources for several parasitoid species and demonstrated that flower attractiveness was not strictly correlated with nectar accessibility. Similarly, Jacob and Evans (2000) reported that adult *B. curculionis* did forage readily on dandelion flowers, but that foraging wasps had difficulty entering into and piercing florets to obtain nectar. In the present study, we observed adult *P. tricuspis* foraging on buckwheat flowers, but apparently with minimal utilization of the nectar. This may suggest that buckwheat florets are morphologically incompatible with the mouthparts of *P. tricuspis*, as has been reported for other insects (Jervis, 1998; Jervis et al., 1993), or the possibility of innate behavioral characteristics of phorid flies that limit nectivory.

It is possible that parasitoids with short mouthparts, such as phorid flies, are likely to utilize exposed sugar sources, such as honeydew and extra floral nectar more

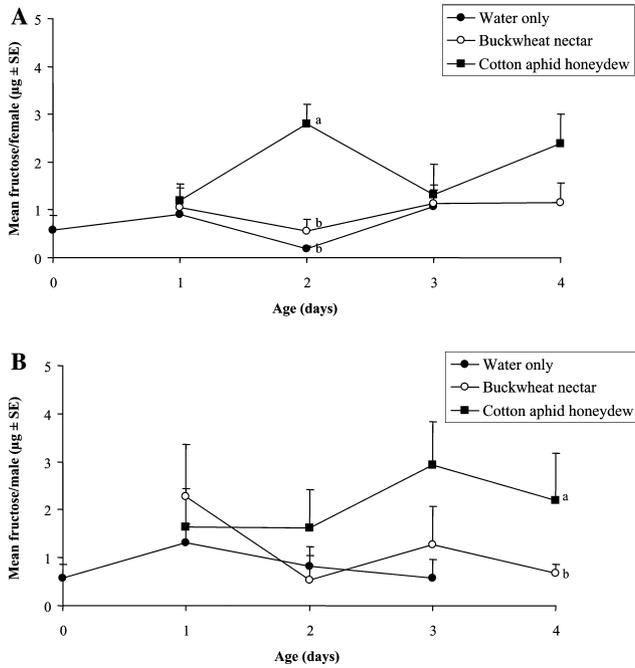


Fig. 2. Mean amounts (microgram ± SE) of fructose in female (A) and male (B) *P. tricuspidis* that were provided water only, buckwheat nectar, or cotton aphid honeydew. Means for the same fly age having different letters are significant (Tukey–Kramer HSD,  $P < 0.05$ ).

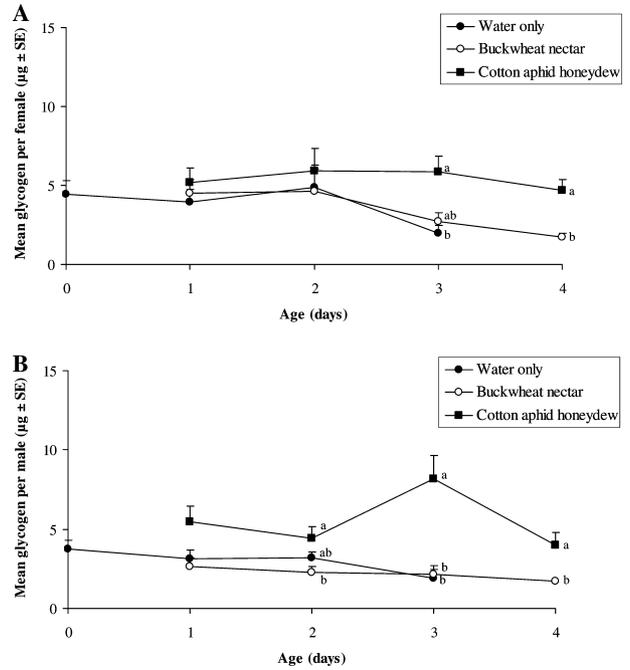


Fig. 4. Mean amounts (microgram ± SE) of glycogen in female (A) and male (B) *P. tricuspidis* that were provided water only, buckwheat nectar, or cotton aphid honeydew. Means for the same fly age having different letters are significant (Tukey–Kramer HSD,  $P < 0.05$ ).

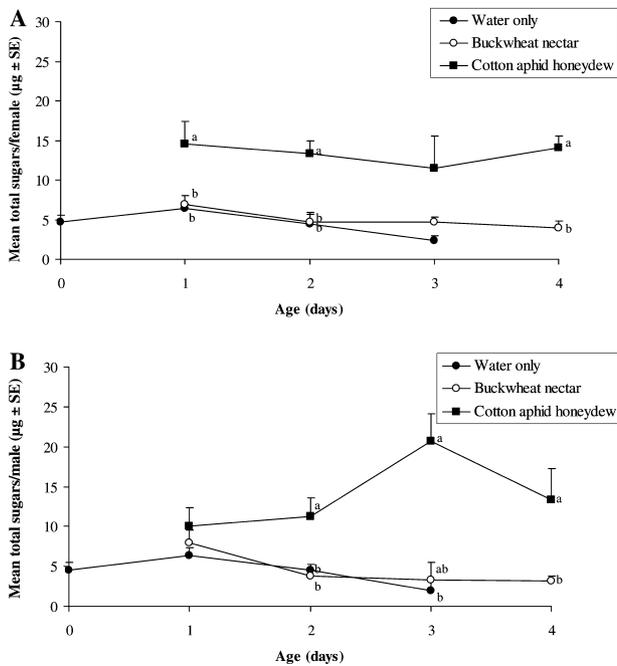


Fig. 3. Mean amounts (microgram ± SE) of total sugars in female (A) and male (B) *P. tricuspidis* that were provided water only, buckwheat nectar, or cotton aphid honeydew. Means for the same fly age having different letters are significant (Tukey–Kramer HSD,  $P < 0.05$ ).

than floral nectar, as sugar sources in the field (Gilbert and Jervis, 1998; Jervis, 1998). Gilbert and Jervis (1998) indeed, reported a strong association between proboscis

length and the type of food sources visited by tachinid parasitoid flies (Diptera: Tachinidae); flies with long (>height of head) or moderately long (= height of head) proboscis tended to visit flowers only, while flies with short proboscis (<height of head) were observed visiting honeydew and extrafloral nectaries, as well as flowers. The authors concluded that the possession of short proboscis in Tachinidae and many other parasitoid flies is likely associated with feeding on honeydew and exposed nectaries. The short and fleshy proboscis with teeth-bearing labella commonly found in many phorid flies (Gilbert and Jervis, 1998) may indeed be an adaptation for collecting honeydew from surfaces, such as leaves (Disney, 1994). Sexual dimorphism in mouthpart structure is also known to occur in Phoridae (Gilbert and Jervis, 1998), however, we did not observe any notable differences in the feeding behavior or effects of specific diets on the lifespan and nutrient levels of female and male *P. tricuspidis*. Apart from mouthpart structure, other morphological structures such as head width may also limit accessibility of floral nectar to parasitoids. Wasps with relatively greater head width will need to possess modified mouthparts with concealed nectar extraction apparatus (CNEA), such as modified labial and maxillary structures in order to obtain nectar from relatively short tubular corollas (Jacob and Evans, 2000; Jervis, 1998). However, it is highly unlikely that the small head width of *P. tricuspidis* (~0.3–0.35 mm wide for males and 0.35–0.4 mm wide for females at the mesonotum) will limit its access to floral nectar.

Alternatively, buckwheat nectar may be quantitatively or qualitatively inferior to cotton aphid honeydew or may contain compounds that are mildly repellent to *P. tricuspis*. Binns (1980) reported that the mushroom phorid fly, *Megaselia halterata* (Wood) (Diptera: Phoridae) preferred certain honeydew sugars, such as sucrose, fructose, and melezitose to lactose, maltose, glucose or D-mannitol. Studies are currently ongoing in our laboratory to compare utilization of specific nectar and honeydew sugars by *P. tricuspis*. Future studies will investigate potential mechanisms behind the observed reduced suitability of buckwheat nectar as a food source for *P. tricuspis* including an analysis of the composition of honeydew and buckwheat nectar, and the foraging mechanisms of phorid flies.

In the present study, cotton aphid honeydew modestly increased phorid fly lifespan by about 1 day with moderate but significant increases in body carbohydrate nutrient levels, whereas feeding on sucrose solution was found in earlier studies to increase longevity by about 4–5 days with a relatively greater effect on body nutrient levels (Chen et al., 2005; Fadamiro et al., 2005). The observed reduced utilization of honeydew by *P. tricuspis* compared to sucrose solution (Chen et al., 2005; Fadamiro et al., 2005) may be related to differences in sugar quality (Baker and Baker, 1983; van Handel et al., 1972; Wäckers, 2001), or more likely, to reduced availability of honeydew solution on cotton leaves due to rapid crystallization of some components of honeydew sugars (Chen and Fadamiro, unpublished data; Wäckers, 2001).

We recorded significant and positive effects of diet and age on body nutrient levels of *P. tricuspis*, but sex and mating status of flies had no effect on their longevity and ability to accumulate body nutrients. The effect of body size as measured by wing length on body nutrient levels was only significant for glycogen levels in males, with larger males accumulating more glycogen from honeydew than smaller males. These results are, in general, consistent with those reported in earlier studies on *P. tricuspis* (Chen et al., 2005; Fadamiro et al., 2005). Also, body nutrient levels obtained in this study were generally similar in range to those previously reported for *P. tricuspis* (Fadamiro et al., 2005), with the exception of the relatively lower glycogen levels. Female and male phorid flies used in the current study had a mean glycogen level of about 4 µg at emergence, compared to the 8.6 and 5.6 µg previously reported for female and male *P. tricuspis*, respectively, under similar test conditions (Fadamiro et al., 2005). The reduced glycogen levels recorded in this study may reflect differences in emergent nutrient reserve levels of different batches of phorid flies. Variations in the emergent levels of nutrient reserves of different batches of phorid flies may be related to size differences at emergence, or to the quality of hosts on which they were reared.

In conclusion, our data demonstrate that adult *P. tricuspis* could potentially utilize aphid honeydew as sugar sources in the field. Feeding on honeydew or other sugar sources may potentially increase the fecundity of *P. tricuspis*, as reported for the mushroom phorid, *M. halterata* (Binns, 1980). In contrast, however, Zacaro and Porter (2003) detected no oocytes in previtellogenic and vitellogenic growth phases in the ovarioles of *Pseudacteon wasmanni* Schmitz, suggesting that oogenesis may occur only during the pupal stage in phorid fly species. Future studies will determine the life history strategy and the potential effects of sugar feeding on fecundity of *P. tricuspis*. Phorid flies may be able to obtain nourishment from certain floral nectars and extrafloral nectaries in the field. However, there is need to identify the flowers that are potentially suitable to *P. tricuspis* in its release areas, and to determine the potential of such foods on phorid fly longevity and reproductive potential. Conservation of naturally occurring sugar sources in the field, such as suitable floral plants, honeydew and other exposed sugar sources, may promote phorid fly lifespan and its potential biological control impact in southern United States.

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