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FECUNDITY AND LONGEVITY OF THE SIBLING AND SYMPATRIC SPECIES, *TRUPANEA NIGRICORNIS* (COQUILLETT), A POLYPHAGE, AND THE NARROWLY OLIGOPHAGOUS *T. BISETOSA* (COQUILLETT) (DIPTERA: TEPHRITIDAE)

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Abstract.—The sibling species, *Trupanea nigricornis* (Coquillett) and *Trupanea bisetosa* (Coquillett) are not sexually mature when they emerge from puparia, and must feed to attain sexual and reproductive maturity. In both species, males matured earlier than females and gonadal maturation was independent of diet. Females of *T. nigricornis* and *T. bisetosa* required an extrinsic source of proteins, in addition to sugar and water, to mature eggs. They showed similar pre-oviposition periods (18–20 d) on protein diets. Omission of vitamins to a protein diet did not inhibit ovarian maturation in both species, but lengthened their pre-oviposition periods. Addition of the host plant to a protein diet did not affect the pre-oviposition period, but prevented egg resorption and led to successful mating. No significant differences were detected in longevity between the two species fed on carbohydrate or protein diets, and between males and females of the same species, with the exception of *T. nigricornis* females fed on yeast hydrolysate diet. When given the choice, females of each species preferred to oviposit in flower heads of its natural host. In non-choice experiments, *T. nigricornis* could not successfully oviposit in the flower heads of wild sunflowers, the host of *T. bisetosa*, but *T. bisetosa* females did oviposit in the non-host heads of *Encelia farinosa*. On their natural hosts, the fecundity of *T. nigricornis* (244 eggs/female) was higher than that of *T. bisetosa* (158 eggs/female). Mating influenced egg production in both species. The fecundity of unmated *T. nigricornis* (14 eggs/female) and *T. bisetosa* (8 eggs/female) females on their host plants, *E. farinosa* and *H. annuus*, respectively, was much lower than the fecundity of mated females on their host plants.

Key Words: adult food, fruit flies, ovarian maturation, survival

Trupanea nigricornis (Coquillett) and *Trupanea bisetosa* (Coquillett) are flower-head infesting tephritids that occur in sympatry in southern California. They are sibling and cryptic species showing great similarities in their morphology, biology and behavior (Knio et al. 1996a, b). Immatures of both species are difficult to separate and can only be recognized by their host plant (Knio et al. 1996a). Adult males can be easily separated by the color of the third antennal

segment, brown in *T. nigricornis* and yellow in *T. bisetosa* However, females are more difficult to identify; about 75% can be recognized by the shape of the Y-apical marking on the wings: short and broad in *T. nigricornis* vs. distinct and thin in *T. bisetosa* (Cavender and Goeden 1983, Foote et al. 1993). Ecologically, the two species show major differences in their mode of herbivory and host plant specificities. *Trupanea nigricornis* behaves as a generalist, infesting plants belonging to 8 tribes of the Asteraceae while *T. bisetosa* is a narrow oligophage, specializing mainly on the flower heads of *Helianthus annuus* L. (Asteraceae).

Resource utilization studies showed that the larvae of both species exploited the flower heads of their hosts in a similar manner and they damaged a similar number of achenes (Knio et al. 2001). Such studies did not provide clues to why these two sister and sympatric species have diverged in their mode of herbivory. Comparisons of the types and densities of natural enemies attacking these two species showed that the generalist, *T. nigricornis*, suffered mortality by more species of parasitoids (5 vs. 1) than the specialist, *T. bisetosa*. Moreover, percentage parasitism was much higher in samples infested with *T. nigricornis* (21.5–58.2%) than *T. bisetosa* (4.5–16.1%). Therefore, it seems that by specializing on wild sunflowers, *T. bisetosa* gained an enemy-free space (Knio et al. 2007). It is not known whether *T. nigricornis* can compensate for the higher mortality caused by parasitoids by having a higher fecundity than *T. bisetosa*. Moreover, studying demographic parameters of the adult populations, such as fecundity and longevity, might shed further light on the different strategies adopted by these two species.

In tephritids, adult survival and reproduction are affected by nutrition and temperature (Hendrichs et al. 1993, Jácome et al. 1999, Vargas et al. 2000). There have been extensive studies on the effect of diets on the longevity and fecundity of fruit-infesting and economic important species, but no information exists on the flower-head infesting species, which cannot be reared in the laboratory on artificial diets. Studies on fruit-infesting tephritids showed that newly emerged adults of most species are not sexually mature and females need, under optimum conditions, several days to maturate eggs. Although adult tephritids differ in their nutritional requirements as there are differences among individuals and species in the amount and quality of metabolites transferred from the larval to the adult stage, most species need an extrinsic source of sugars and water for survival, and regular ingestion of proteins, vitamins and minerals for adequate egg production (Tsiropoulos 1977, 1978, 1980). In nature, food sources for adult fruit flies included damaged fruits, plant exudates, plant sap, pollen, nectar, honey dew, microorganisms, and bird droppings (Bateman 1972, Fletcher 1987, Hendrichs et al. 1993).

Relatively few demographic and life history studies have been conducted on the flower-head infesting tephritids, and more specifically on *T. nigricornis* and *T. bisetosa*. The pattern of reproduction and effect of adult nutrition on survival and fecundity have not been studied in those species. In this study, we aim to compare the influence of different diets, including the host plants of *T. nigricornis* and *T. bisetosa*, on gonadal maturation, and longevity of the adults, and to determine the fecundities of *T. nigricornis* and *T. bisetosa* females on host and non-host plants. The effect of mating on fecundity is also investigated. These life history studies constitute one of a series intended to shed light on the nature of polyphagy/oligophagy in the sibling and sympatric species, *T. nigricornis* and *T. bisetosa*.

Materials and Methods

Study insects.—*Trupanea nigricornis* adults were reared from *Encelia farinosa*

Gray (Asteraceae) heads while *Trupanea bisetosa* adults were reared from flower heads of wild sunflowers, *Helianthus annuus* L. (Asteraceae) collected from four interior valley sites in southern California. The collected flower heads were placed in separate glass-topped, sleeve cages (34×32×35 cm) in the insectary at 60% RH and a 12/12 h LD photoperiod from 0500–1700 h to monitor for the emergence of adult tephritids. Newly emerged (one day old) adults were used for the following experiments.

Effect of diets on ovary maturation.—*Effect of sugar, proteins and host:* Insectary cages were provisioned with six different diets: (1) control diet: water and honey (as a source of carbohydrates mainly); (2) water, honey, and flower heads of *E. farinosa* for *T. nigricornis* or *H. annuus* for *T. bisetosa*; (3) water, honey and casein hydrolysate (as a source of proteins mainly); (4) water, honey, casein hydrolysate and *E. farinosa* flower heads; (5) water, honey, and yeast hydrolysate (as a source of proteins mainly); and (6) water, honey and yeast hydrolysate and *E. farinosa* flower heads.

Yeast and casein hydrolysates, two commonly used, rich protein diets on fruit-infesting species, were tried as extrinsic source of proteins for *T. nigricornis*. Since they both gave similar results, only yeast hydrolysate as protein diet was adopted for *T. bisetosa*. Therefore, only diets (1), (2), and (5) were tested for *T. bisetosa* adults.

The yeast and casein hydrolysate solutions were prepared by mixing the protein hydrolysate: sucrose: water in the ratio of 4: 7: 10 (Tsiropoulos 1978). Tightly wrapped, absorbent sterile cotton wicks (ca 2×1 cm) were dipped in the protein solutions and hung from the glass top of the sleeve cage with scotch tape. The cotton wicks were changed every other day. On the second day, they were moistened with ca. 1 ml of distilled water added with a pipette. The immature flower heads of *E. farinosa* and *H. annuus* were attached to stems which were immersed in water. They were changed every 2–3 d. In each cage, honey was streaked on the underside of the glass and water was provided in a glass bottle with a cotton wick. Every cage initially contained 40 females and ten males newly recovered (one day old) from the flower heads. Each treatment was replicated twice. Subsamples of three females (six females per treatment) were taken from each cage 5, 10, 15, 20, 30 and 50 d after emergence. The females were chilled for 30 min then placed in 70% ethanol and dissected under a stereomicroscope to examine the condition of the ovaries. Ovary size was measured with an ocular micrometer. The preoviposition periods of the females given different diets were estimated by monitoring the presence of mature eggs in (i) dissected ovaries, and (ii) in flower heads of *E. farinosa* or *H. annuus*. For the latter step, three females were taken at each sampling interval from the cages provided with diets lacking the host plant, and placed for 3–4 h in Petri dishes (100×15 mm) with excised immature *E. farinosa* or *H. annuus* flower heads to allow oviposition. The females were not returned to their cages to avoid variables caused by probing or feeding on the flower heads.

Effect of vitamins: To separate the effect of protein and vitamins on ovarian maturation, newly emerged (one day old) flies were fed on a diet of water, sucrose and protein devoid of vitamins (Vitamin Assay Casamino Acids dehydrated, Difco Laboratories, Detroit, Michigan) (10: 7: 4). Newly emerged adults of both species were placed in separate cages (40 females and 10 males per cage), as described above. The control diet was yeast hydrolysate, sucrose, and water. A subsample of six females was taken from each treatment at day 10, 15, 18, 20, 25, 30, 35, and 50 after adult emergence. The

females were dissected and the size and conditions of the ovaries were recorded as described above.

Effect of diets on male gonadal maturation.—Newly emerged adults (one day old) of *T. nigricornis* (20 females and 20 males) and *T. bisetosa* (20 females and 20 males) were paired, and each pair was placed in a small plastic cage. For every species, 10 pairs were offered a diet of water and honey, and 10 other pairs were offered water, honey and yeast hydrolysate. The first time a male started to exhibit courtship display was recorded.

Effect of diets on adult longevity.—Newly emerged adults (one day old) of *T. nigricornis* and *T. bisetosa* were divided into three subsamples and provided with the following diets: (1) water, (2) water and honey, (3) water, honey and yeast hydrolysate diet, (4) water, honey, yeast hydrolysate and immature flower heads of *E. farinosa* or *H. annuus*, depending on the tephritid species. For every treatment, male and female flies of the same species were placed in the same cage. Longevity of the flies under these different diets was determined by recording, daily, the date of natural death.

Fecundity.—*Initial experiments (group fecundity):* Initial fecundity experiments were carried out to determine whether *T. nigricornis* and *T. bisetosa* would oviposit under artificial conditions in host and non-host flower heads and to determine the stage of the flower heads suitable for oviposition. Newly emerged adults of both species were placed in separate glass-topped sleeve cages (34×32× 35 cm) in the insectary and given a yeast hydrolysate diet, honey, and water. Adults of each species were divided into three subsamples of 20 flies. Adults in the first subsamples were provided with bouquets of immature *H. annuus* flower heads. Adults in the second subsamples were provided with *E. farinosa* flower heads; those in the third subsamples were provided with *E. farinosa* and *H. annuus*. The flower heads were changed every three days and dissected under a stereomicroscope to determine whether oviposition had occurred. The time the females started to oviposit was also recorded as well as the plant host that was preferred by each species under laboratory conditions.

Individual fecundity experiment: One-day old, sexed adults were given yeast hydrolysate, honey and water. At day 18 after emergence (as determined from the previous experiments on the effect of diets on timing of flies' sexual maturity), the flies were paired (female × male of *T. nigricornis* or female × male of *T. bisetosa*) and transferred to a smaller cage: 850 ml clear plastic cage with a basal water reservoir and a screened lid for ventilation (Cavender and Goeden 1983). The lid of each cage was striped with honey and pinned with a cotton wick dipped in the yeast hydrolysate diet. Each cage was provided with a bouquet of immature flower heads, the peduncles of which were immersed in the basally attached water reservoir and held in place by a moist absorbent cotton plug that also provided a water source for the flies. To study the flies' fecundity on their host plants, adults of *T. nigricornis* were provided with bouquets of *E. farinosa* (open buds). Adults of *T. bisetosa* were provided with bouquets of sunflowers (very small closed buds). To test the fecundity of these flies on the other species host plants, *T. nigricornis* adults were provided with bouquets of wild sunflowers (very small closed buds) while *T. bisetosa* adults were provided with bouquets of *E. farinosa* (open buds). To be certain that the flower heads collected were not infested, the immature heads were previously covered with a fine-mesh cloth to prevent fly from oviposition or they were collected while free of oviposition wounds and placed in water at room

Table 1. Pre-oviposition period and ovarian size of *Trupanea nigricornis* females on different diets.

Days After Emergence	Mean Ovary Size (Diameter × Length in mm; n = 6) of Females Given[a]:					
	W+H	W+H+Y	W+H+Y+F	W+H+F	W+H+C	W+H+C+F
5	0.18×0.23	0.23×0.36	0.21×0.32	0.16×0.25	0.21×0.32	0.19×0.29
10	0.18×0.27	0.23×0.38	0.24×0.38	0.17×0.27	0.24×0.43	0.3×–0.4
15	----------	----------	**0.42×0.72**[c]	----------	----------	----------
20	0.18×0.26	**0.43×0.79**[b]	**0.56×0.93**	0.18×0.3	**0.5×0.86**	**0.49×0.92**
30	0.18×0.27	**0.46×0.77**	**0.59×0.97**	**0.62×1.04**	**0.52×0.87**	**0.57×1.01**
50	0.21×0.33	**0.53×0.84**	**0.52×0.99**	**0.5×0.7**	**0.49×0.87**	**0.51×0.79**
Pre-oviposition Period	----------	20	15–20	30	20	20

[a] Diets: water and honey (W+H) (control); yeast hydrolysate diet (Y); casein hydrolysate diet (C); *Encelia farinosa* flower heads (F).
[b] The presence of mature eggs in the ovaries of all sampled females is indicated in bold face.
[c] Only in this sample, three out of six females had mature ova in their ovaries.

temperature for 2 d before using them, so that any eggs still present would have shriveled if infertile or hatched into first instars. The bouquets of the immature flower heads were changed every 2–3 d, and then dissected under a stereomicroscope to determine the number of eggs laid. All the eggs that were found were placed on a filter paper (Whatmann #1) moistened with physiological saline in closed glass Petri dishes, and incubated at 27°C. The eggs were checked every day for eclosion.

Effect of mating on fecundity: To study the effect of mating on fecundity, individual newly emerged *T. nigricornis* (n = 10) and *T. bisetosa* (n = 10) females were placed in small cages and given a yeast hydrolysate diet as described above. At day 18 after emergence, *T. nigricornis* females were provided, every 3 d, with a bouquet of *E. farinosa* flower heads while *T. bisetosa* females were provided, every 3–4 d, with wild sunflower heads. The number of eggs oviposited by each female and percentage egg hatch were recorded as described above. This data was then compared and plotted against the number of eggs oviposited by *T. nigricornis* and *T. bisetosa* females from the individual fecundity experiments.

Statistics.—Statistical differences in the mean timing of reaching sexual maturity of *T. nigricornis* and *T. bisetosa* males fed on yeast hydrolysate or water and honey were tested by performing two-tailed *t*-tests for independent variables. Tukey's test for pairwise comparison of means was conducted to test differences between average longevity of *T. nigricornis* and *T. bisetosa* adults on different diets tested. All statistical tests were performed at 95% confidence level using SPSS (13.0).

Results

Effect of diets on ovary maturation.—*Effect of sugar, proteins and host:* The conditions of the ovaries and pre-oviposition periods of *T. nigricornis* and *T. bisetosa* females given different diets are summarized in Tables 1 and 2. The control diet, which was honey and water, did not support ovary maturation in either species. Honey, which is composed of a high proportion of sugars, of which 40–50% is sucrose, water, and traces of proteins (Chapman 1982), provided mainly a source of carbohydrates. Sugars alone were not sufficient to support egg production in *T. nigricornis* and *T. bisetosa* females. Thus, the ovaries in females of neither species matured on

Table 2. Pre-oviposition period and ovarian size of *Trupanea bisetosa* females on different diets.

Days After Emergence	Mean Ovary Size (Diameter × Length in mm; n = 6) of Females Given[a]: (W+H)	(W+H+Y)	(W+H+Y+F)
5	0.17×0.24	0.19×0.39	0.20×0.30
10	0.20×0.28	0.24×0.33	0.25×0.36
15	0.20×0.27	0.26×0.52	----------
20	0.18×0.30	**0.51×0.73**[b]	**0.50×0.75**
40	0.22×0.30	**0.42×0.65**	**0.55×0.83**
50	0.20×0.30	**0.43×0.65**	**0.50×0.70**
Preoviposition Period	----------	20	20

[a] Diets: water and honey (W+H) (control); yeast hydrolysate diet (W+H+Y); excised sunflower heads (F).

[b] The presence of mature eggs in the ovaries of all sampled females is indicated in bold face.

a sugar and water diet, and the follicles in the immature ovaries became resorbed between 40 and 50 d after female emergence. The resorbed ova were translucent and yellowish.

All protein hydrolysate diets tested supported ovigenesis in *T. nigricornis* females. With all these diets, the ovaries matured, and they each contained six to eight mature ova (0.20 mm in diam.; 0.70 mm in length). There was no difference in the diets containing casein or yeast hydrolysates, with or without the host plant, with respect to the pre-oviposition period, which was about 20 d for all the protein diets tested (Table 1). Thus, both casein and yeast hydrolysate provided the essential nutrients required for the production of mature eggs.

The addition of the host plant to the protein hydrolysate diet did not affect the pre-oviposition period of *T. nigricornis* females, but it affected two phenomena: egg resorption and mating. When *T. nigricornis* adults were fed on protein diets but were not offered flower heads of their hosts, most (90%) of the females had the ova in their ovaries resorbed by days 40 and 50, as they had no access to oviposition sites.

Egg resorption did not occur in *T. nigricornis* and *T. bisetosa* provided with protein diets and flower heads of their hosts. At day 50 after emergence, the females that were given protein diets and *E. farinosa* heads contained expanded, empty ovaries, resembling two large, opaque sacs (mean of four empty ovaries: 0.66 mm in diameter; 1.21 mm in length). These females had laid most of the eggs produced in their ovaries. Also, the presence of the host plants, together with the protein diet, provided cues for mating, since successful matings were only recorded in the cages provided with protein hydrolysate and *E. farinosa* heads.

Similar to *T. nigricornis*, the females of *T. bisetosa* also required a protein source for their ovaries to mature (Table 2). They developed mature ova on day 20 on yeast hydrolysate diets, with or without their host plant (Table 2).

The diet comprising water, honey, and *E. farinosa* heads also supported ovigenesis in *T. nigricornis* females. Females showed mature ova on this diet at day 30 compared to day 20 with the protein hydrolysate diets (Table 1).

Effect of vitamins: The absence of vitamins in the diet did not inhibit ovarian maturation in *T. nigricornis* and *T. bisetosa*, but affected their pre-oviposition period (Table 3). Both *T. nigricornis* and *T. bisetosa* females matured eggs when fed proteins devoid of vitamins, sugar, and water. The pre-oviposition period, however, varied among females; not all sampled females

Table 3. Ovarian maturation and pre-oviposition period of *Trupanea nigricornis* and *Trupanea bisetosa* with protein alone, sucrose, and water diet (A) and with yeast hydrolysate diet (control) (B).

Days After Emergence	Number of Mature Females[a] from n = 6:			
	T. nigricornis		*T. bisetosa*	
	A	B	A	B
10	0	0	0	0
15	0	0	0	0
18	0	5 (83.3%)	0	4 (66.7%)
20	2 (33.3%)	6 (100%)	2 (33.3%)	6 (100%)
25	1 (16.7%)	6 (100%)	3 (50%)	6 (100%)
30	2 (33.3%)	6 (100%)	3 (50%)	6 (100%)
35	6 (100%)	6 (100%)	6 (100%)	6 (100%)
50	6 (100%)	6 (100%)	6 (100%)	6 (100%)

[a] A female was considered mature if the ovaries contained fully developed eggs.

reached reproductive maturity during the same week after emergence (Table 3). Few females of both species had mature ova in their ovaries at days 20 and 30 after emergence. All females matured ova at day 35 after emergence compared to day 20 with yeast hydrolysate (control) diet (Table 3).

Effect of diets on male gonadal maturation.—*Trupanea nigricornis* and *T. bisetosa* males reached sexual maturity about 5–10 d after emergence: 7.2 ± 0.4 (5–10) days for *T. nigricornis* (n =10) and 8.2 ± 0.4 (6–10) days for *T. bisetosa* (n = 10). No difference in the mean timing of reaching sexual maturity was detected for *T. nigricornis* and *T. bisetosa* males fed on yeast hydrolysate or water and honey ($t = 0.73$; $P > 0.05$ and $t = 0.35$; $P > 0.05$, respectively). Unlike the females, the males did not need an extrinsic source of nitrogen for gonadal development since they became mature on the yeast hydrolysate diet as well as the carbohydrate diet, and at the same time.

Effect of diets on adult longevity.—*Trupanea nigricornis* and *T. bisetosa* adults survived in the insectary for 2–3 months on the diets tested, including the water and honey diet. They survived on water alone for only 2–4 d (Table 4). This shows that at least carbohydrates and water are essential for survival. The role of proteins in prolonging longevity is not very clear, as there was little difference in longevity between the carbohydrate and protein diets for both species (Table 4). Only the females of *T. nigricornis* showed a significantly greater mean longevity on yeast hydrolysate diet than on water and honey diet. There was no significant difference in longevity between male and female flies for either species fed on yeast hydrolysate or water and honey diet, with the exception of *T. nigricornis* females on the yeast hydrolysate diet, which survived longer than the males (Table 4). Hence, both *T. nigricornis* and *T. bisetosa* survived for a period of 2–3 months, and sometimes up to 4 months on diets of yeast hydrolysate or honey and water in the insectary.

When *T. nigricornis* and *T. bisetosa* females were fed on yeast hydrolysate diets, but were also given flower heads of their host for oviposition, they lived a maximum of 2 months (Table 4). The longevity of ovipositing females was significantly lower than that of females deprived of oviposition sites, but fed on the same protein hydrolysate diet (Table 4). This could be due to the exhaustion of nutrients and energy needed for continual egg production and egg-laying in the ovipositing females.

Unlike the females, the longevity of *T. nigricornis* and *T. bisetosa* males did not

Table 4. Longevity (in days) of *Trupanea nigricornis* (T. n.) and *Trupanea bisetosa* (T. b.) on different diets.

Diet[a]	Species	N	Mean[b] Longevity ± SE (range)	
			Males	Females
W	T. n.	10	2.6 ± 0.27a (1–4)	2.9 ± 0.22a (2–4)
	T. b.	10	2.5 ± 0.26a (1–4)	2.4 ± 0.24a (1–3.5)
W+H	T. n.	40	87.4 ± 2.3c (65–116)	91.6 ± 2.6c (60–118)
	T. b.	15	84.5 ± 3.42c (65–102)	87 ± 3.5c (65–102)
W+H+Y	T. n.	40	89.1 ± 2.7c (65–120)	98.4 ± 3.2d (65–124)
	T. b.	20	91.1 ± 3.7c (65–120)	95.3 ± 4c, d (68–122)
W+H+Y+F	T. n.	17	84.8 ± 4.1c (64–124)	64.7 ± 4.2b (30–86)
	T. b.	17	86.6 ± 3.4c (58–119)	65.6 ± 4.3b (30–88)

[a] Diet: water (W); water and honey (W+H); water, honey and yeast hydrolysate (W+H+Y); water, honey, yeast hydrolysate and flower heads of *Encelia farninosa* for *T. nigricornis* or sunflowers for *T. bisetosa* (W+H+Y+F).

[b] Means followed by the same letter were not significantly different using Tukey's test at 95% confidence level.

change on protein hydrolysate diet with or without flower heads of their host plants (Table 4). The presence of the flower heads did not change the behavior and activities of the males that exhibited their courtship behavior in the presence and the absence of their host plants.

Fecundity.—*Initial experiments (group fecundity):* Initial experiments showed that the females of *T. nigricornis* oviposited only on their host, *E. farinosa*, and that the females of *T. bisetosa* preferred to oviposit on their host, *H. annuus*, but could also oviposit on the non-host, *E. farinosa* under artificial conditions. Females of both species started to oviposit 16–20 d after emergence. When given a choice between host and non-host flower heads, the females of *T. bisetosa* oviposited in wild sunflower heads if they were at the right stage for oviposition (very small 'closed' buds'). If the sunflower heads were in a stage barely suitable for oviposition (medium 'closed' buds), they preferred to oviposit in the non-host, *E. farinosa*. When given the choice, the females of *T. nigricornis* only oviposited in the flower heads of their host, *E. farinosa*; they did not attempt to oviposit in wild sunflower heads. In non-choice situations, only a few females of *T. nigricornis* attempted to oviposit in sunflower heads.

Individual fecundity experiment: Fecundity studies showed that the females of *T. nigricornis* oviposited a large number of eggs in the flower heads of their host plant, *E. farinosa*, during their life span (ca. 2 months in the insectary). The mean number of eggs laid per 15 females during a period of 8 weeks was 243.7 ± 16.1 (range: 147–377) (Fig. 1). The mean number of eggs laid during the first week was very high (73), then declined progressively to reach a mean of 3.5 eggs during the eighth week (Fig. 2A). The oviposition curve showed a peak during the first week, then a smooth decline with time.

The fecundity of *T. bisetosa* on its host plant, *H. annuus*, was significantly lower than that of *T. nigricornis* on its host plant. The mean number of eggs oviposited by 10 *T. bisetosa* females was 157.8 ± 21.8 (range: 89–268) (Fig. 1). The oviposition curve showed a high peak in the first week (mean of 35 eggs/female), followed by a slow decrease in the number of eggs laid (18–24 eggs/female/week) during weeks 2 to 6, and

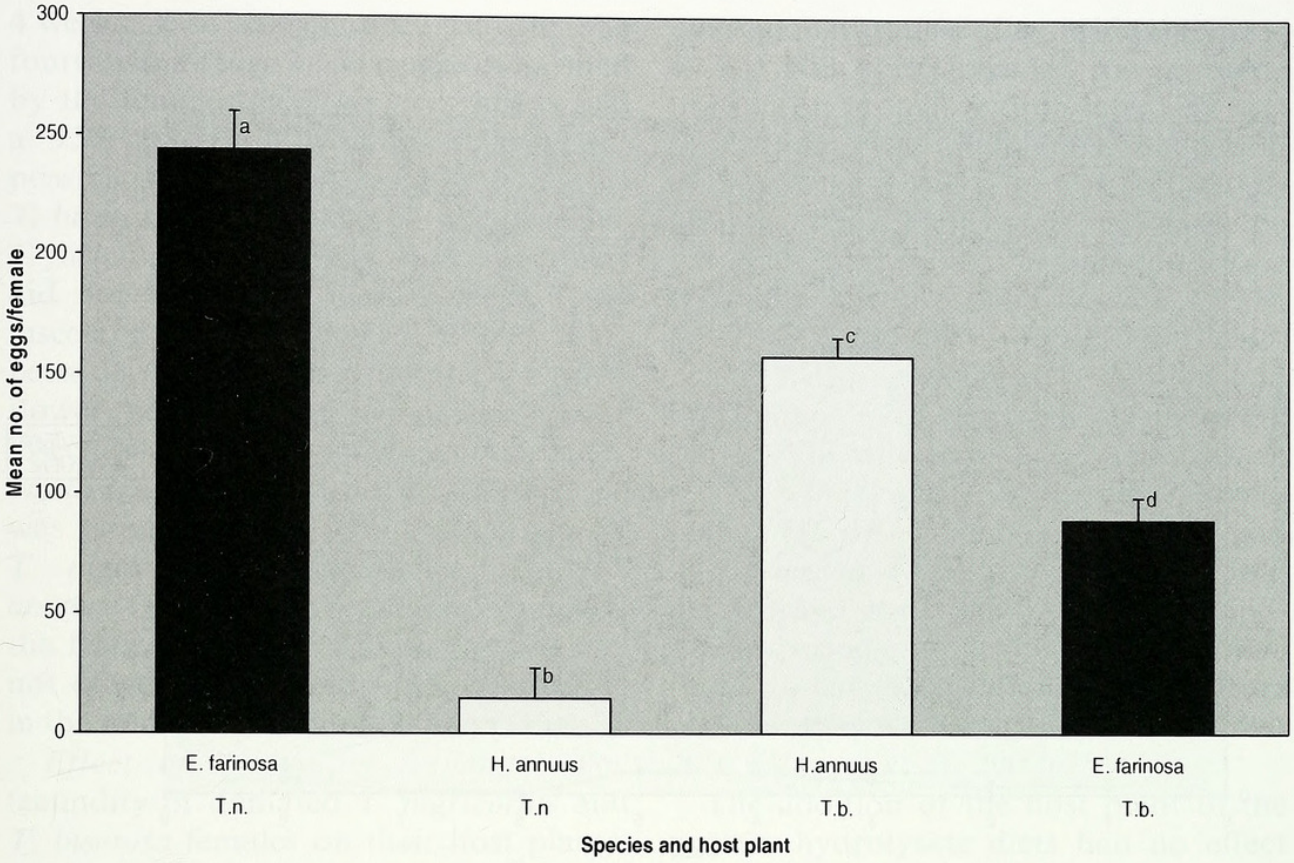

Fig. 1. Fecundity of *Trupanea nigricornis* (T. n.) females on their host plant, *E. farinosa*, and non-host plant, *H. annuus*, compared to the fecundity of *T. bisetosa* (T. b.) females on their host plant, *H. annuus*, and the non-host plant, *E. farinosa*.

a sharp decline in oviposition after week 6 to reach a mean of 5.1 eggs during the eighth week (Fig. 2B).

The fecundity of *T. nigricornis* females on the non-host flower heads of wild sunflowers was very low. The mean number of eggs oviposited by 10 females in 8 weeks was 12.6 ± 6.4 (range: 0–64) (Fig. 1). This mean was significantly lower than the mean number of eggs laid by *T. bisetosa* on the non-host plant, *E. farinosa*. Four *T. nigricornis* females did not lay eggs on the non-host flower heads of *H. annuus*. The other females did not lay any eggs in the first week, then started ovipositing a few eggs as they do in their host plant heads, i.e., by piercing the plant tissues. However, the females of *T. nigricornis* could not successfully oviposit in sunflower buds, which, unlike *E. farinosa* heads, are covered by hard bracts and exude much resins when pierced. Instead of placing the eggs underneath the bracts and among the florets (Knio et al. 1996b) like *T. bisetosa* females do, the females of *T. nigricornis* pierced the hard tissues of the outer bracts while ovipositing, inserting the basal end of the egg in the tissues of the outer bract. This manner of placing the eggs prevented the first instar from hatching, as the basal part of the egg was covered with resins; it also caused the exposed eggs to dry quickly, and resulted in the death of some females when their ovipositors and hind legs became trapped in the exuding resins.

Oviposition by *T. bisetosa* in the non-host flower heads of *E. farinosa* was significantly less than oviposition on their host plant. The females laid a mean number of 89.5 ± 9.4 (58–145) eggs during a period of 8 weeks (Fig. 1). They did not oviposit every day. Plots of the intervals of time (2–5 d) between ovipositions declined slowly during the first

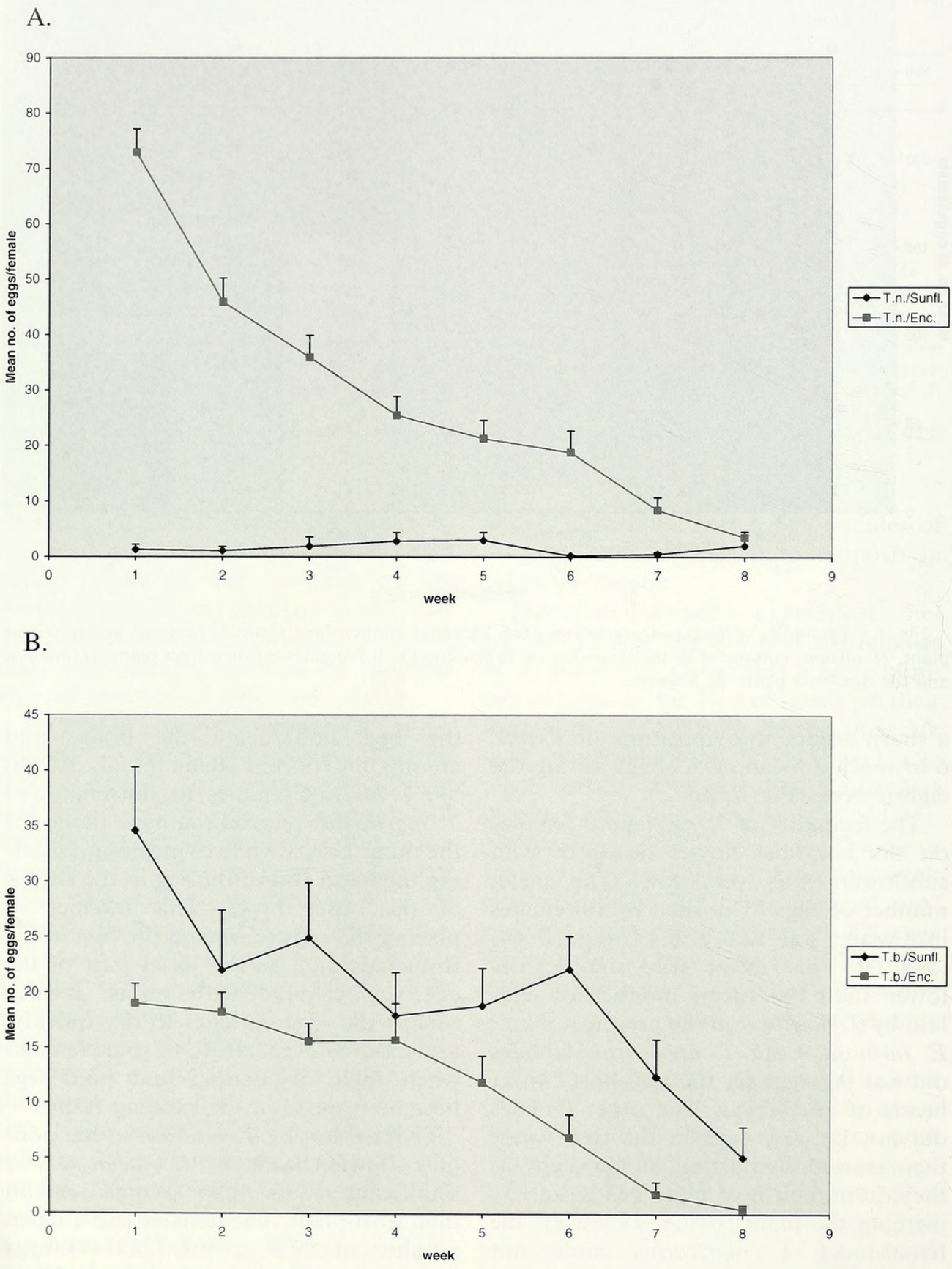

Fig. 2. Fecundity curves, showing the mean number of eggs (± SE) laid per female (n = 15) per week, during a period of 8 weeks, for: (A) *T. nigricornis* (T. n.) on *E. farinosa* (Enc.) and the non-host, *H. annuus* (Sunfl.); (B) *T. bisetosa* (T. b.) on *H. annuus* and the non-host, *E. farinosa*.

4 weeks; then sharply declined after the fourth week (Fig. 2). The eggs oviposited by the females in *E. farinosa* heads had a 95% eclosion; however, it was not possible to know whether the larvae of *T. bisetosa* could develop in the heads of *E. farinosa* as excised *E. farinosa* bouquets did not last more than a week in the insectary and no artificial diet has yet been developed for rearing the larvae of flower head-infesting tephritids. Under insectary conditions, oviposition by *T. bisetosa* in the non-host plant, *E. farinosa* was more successful than oviposition of *T. nigricornis* on non-host plant, *H. annuus*. Unlike *T. nigricornis* females, the fecundity of *T. bisetosa* females was not drastically reduced when ovipositing in the non-host plant, *E. farinosa* (Fig. 2).

Effect of mating on fecundity: The fecundity of unmated *T. nigricornis* and *T. bisetosa* females on their host plants, *E. farinosa* and *H. annuus*, respectively, was much lower than the fecundity of mated females on their host plants. The mean total number of eggs laid by 10 unmated females *T. nigricornis* was 13.8 ± 1.2 (8–20) during a period of eight weeks. The same mean for 8 females of *T. bisetosa* was 8.0 ± 1.5 (4–17). Thus, unmated females of both species did mature and oviposit eggs, but the eggs did not hatch and the fecundity of the females was greatly reduced.

Discussion

The data showed that newly emerged adults of *T. nigricornis* and *T. bisetosa* were not sexually mature, but must feed before attaining reproductive and sexual maturity. Carbohydrate diet did not support egg production in *T. nigricornis* and *T. bisetosa*. Females of both species needed an extrinsic source of proteins for egg maturation.

Both *T. nigricornis* and *T. bisetosa* had matured ova on day 20 when fed protein hydrolysate diets, which contain vitamins and peptides, both essential for ovarian maturation of tephritids (Fytizas 1973). This period needed for egg maturation on protein hydrolysate diets was shorter than the pre-oviposition period of *Rhagoletis completa* Cresson (30 d) (Tsiropoulos 1978), but longer than the period reported for *Bactrocera oleae* (Gmelin) (5–6 d) (Tsiropoulos 1977), (4 d) (Fytizas 1973); *Rhagoletis pomonella* (Walsh) (ca. 10 d) (Webster and Stoffolano 1978, Hendrichs et al. 1993), (6–12 d for wild females) (Webster et al. 1979), *Anastrepha serpentina* (Wiedemann) (16 d) (Jácome et al. 1999), and *A. sororcula* (Zucchi) (14–16 d) (Joachim-Bravo et al. 2003). The pre-oviposition periods of tephritids were also found to vary with different temperature regimes; they were longer at 24°C than at 29°C (Vargas et al. 2000).

The addition of the host plant to the protein hydrolysate diets had no effect on the pre-oviposition period of the flies but prevented egg resorption. Ova resorption was also observed in other flower head infesting tephritids such as *Trupanea conjuncta* (Adams) and *Eutreta simplex* Thomas when deprived of oviposition sites in the insectary (Goeden 1987, 1990). Apparently, this phenomenon helps conserve egg metabolites while the female continues searching for suitable oviposition sites (Goeden 1987).

Females of *T. nigricornis* also matured ova when kept on a sugar diet but were offered *E. farinosa* flower heads. Apparently, they were able to get some sources of vitamins and nitrogen from heads of their host plant, either by feeding on the plant sap flowing from oviposition wounds, on secretions from insects like aphids or thrips that fed on the flower heads, from microorganisms on the flower head surface, or from a combination of these. The amount of nutrients offered by these plant parts was probably low, so that the females needed a longer pre-oviposition period than when they were given protein hydrolysates.

Absence of vitamins in a protein hydrolysate diet did not affect egg maturation of *T. nigricornis* and *T. bisetosa* but resulted in a longer and varied pre-oviposition period. The role of vitamins in the development of the reproductive system of tephritid females is not clearly defined, partly because some transfer of vitamins can occur from the larval to the adult stage (Tsiropoulos 1978, 1980). This could help to explain the variability in the time of ovarian maturation observed in *T. nigricornis* and *T. bisetosa*, because accumulated vitamin reserves obtained from the larval stage could vary among individuals. Similarly, in the walnut husk fly, *Rhagoletis completa*, omission of different vitamin groups was found to influence the pre-oviposition period differently. Omission of vitamin B–mixture or vitamin E did not change the pre-oviposition period, but omission of vitamin C lengthened this period. Both vitamin B-mixture and vitamin C were necessary for fertility in the walnut husk fly (Tsiropoulos 1978).

The effects of diet and proteins on ovarian maturation have been studied almost exclusively in the fruit infesting tephritids. Similar to *T. nigricornis* and *T. bisetosa*, most female tephritids have been found to be anautogenous, requiring an extrinsic source of proteins for ovarian maturation. Carbohydrate diets failed to support egg production by *Bactrocera tryoni* (Froggatt) (Drew 1987), *R. completa* (Tsiropoulos 1978), and *R. pomonella* (Hendrichs et al. 1993). On the other hand, a few species such as *B. oleae*, *A. serpentina* and *C. capitata* could achieve a low fertility when kept on a sucrose diet (Jácome et al. 1999; Tsiropoulos 1977, 1980; Fytizas 1973). This low egg production could have been facilitated by metabolites transferred from the larval stage, and possibly by nutrients synthesized by symbionts (Jácome et al. 1999; Tsiropoulos 1977, 1980). In all cases, protein ingestion in the adult phase significantly increased egg production (Jácome et al. 1999, Cangussu and Zucoloto 1995, Tsiropoulos 1977). However, this increase in fertility in *B. oleae* occurred only when minerals were also added to the protein diet (Tsiropoulos 1980). Analysis of the nutritional requirements of the walnut husk fly revealed that carbohydrates were necessary for the utilization of the dietary proteins and salts were required for protein and vitamin utilization (Tsiropoulos 1978). Furthermore, amino acids have been found to act as phagostimulants for adult tephritids, and those that stimulated feeding by the females proved to be nutritionally necessary for egg production (Tsiropoulos 1986).

The sources of nutrients required for reproduction and survival of fruit flies in the field have been little documented. *Trupanea nigricornis* and *T. bisetosa* were observed feeding on sap exuding from oviposition wounds, honey dew, pollen, and most probably microorganisms found on the flower-head surfaces, as the flies have been observed to constantly extend and retract their proboscis while slowly walking on flower heads. In general, natural food sources of tephritids include fruit juices and pulp, plant exudates, plant sap, pollen, nectar from flowers, honey dew, bacteria and bird droppings (Bateman 1972, Fletcher 1987, Hendrichs et al. 1993). In *R. pomonella*, bird droppings and honey dew as adult food sources resulted in a moderate egg production (Hendrichs et al. 1993). Honey dew lacks some essential amino acids and other nutrients needed for high egg production in tephritids (Hagen and Tassan 1972). Bacteria, on the other hand, offer an important source of proteins to adult tephritids. In *B. tryoni*, adults ingested non-symbiotic bacteria from fruit surfaces, and diets consisting of bacteria, sugar and water resulted in an increased

fecundity comparable to yeast hydrolysate diets (Drew et al. 1983, Courtice and Drew 1983). These bacteria colonizing the alimentary canal of *B. tryoni* belonged to the Enterobacteriaceae, and they were spread during feeding by regurgitation and reingestion processes. They were isolated from fruit surfaces, oviposition wounds, and larval induced fruit rots, but not from leaf surfaces, indicating that these bacteria can be considered as potential food for adults flies (Drew and Lloyd 1987). As for the 'invisible' substances that tephritids are commonly seen grazing on leaves, they are nutrient leachates, mainly carbohydrates, found on the upper surface of foliage and seem important in sustaining survival of the flies (Hendrichs et al. 1993).

Unlike the females, *T. nigricornis and T. bisetosa* males did not need to feed on proteins to reach sexual maturity. Similarly, males of *Bactrocera* sp. required little or no protein for gonadal maturation (Drew 1987). Reproductive maturation of *R. pomonella* males was also independent of diet; the males required little protein for accessory gland development (Webster and Stoffolano 1978). However, in *C. capitata* males, protein nutrition following adult eclosion was found to enhance male maturation and reproductive success (Blay and Yuval 1997, Yuval et al. 2002). When wild *C. capitata* males were fed on a yeast hydrolysate diet, they called more frequently and started sexual calling before those fed on sugar alone (4 vs. 6 d) (Papadopoulos et al. 1998). Protein-fed males were more likely to join leks, emitted more pheromones, and mated more frequently than sugar fed males (Blay and Yuval 1997, Kaspi and Yuval 2000).

Similar to other tephritids, the males of *T. nigricornis* and *T. bisetosa* matured earlier than the females. Two weeks after emergence, they were seen expanding their abdominal pleura and displaying courtship behavior toward the unreceptive females. Male tephritids, in general, become sexually mature earlier than the females (Williamson 1989). Males of *R. pomonella* had active sperms after emergence and became sexually mature 5 d after emergence while females had mature oocytes 10 d after emergence (Webster and Stoffolano 1978). Males of the olive fruit fly also matured 1 to 2 d before the females. Laboratory reared males became sexually mature 3–5 d after emergence, while field-collected males matured 4–15 d after emergence (Zervas 1983). In *C. capitata*, significant differences were also detected in the timing of sexual maturation of wild and laboratory-reared flies. Greater mating activity occurred between 3–5 d after emergence in laboratory reared flies vs. 7–13 d after emergence in wild flies (Liedo et al. 2002). This earlier sexual maturation of laboratory reared flies was also observed in females of *A. suspensa* (Loew). Maturation of oocytes was earlier in mass-reared flies, followed by semi-wild flies adapted for a year to laboratory conditions, and then by wild flies. Moreover, in the semi-wild and wild females, male presence was found to accelerate ovarian maturation (Pereira et al. 2006).

Adults of *Trupanea nigricornis* and *T. bisetosa* survived 2–3 months on the different diets tested, but just a few days on water alone. Carbohydrates were essential for their survival. Similarly, adult nutrition has been found to influence survival of fruit infesting tephritids. Two components essential for tephritid diets were carbohydrates and water (Tsiropoulos 1978); sucrose, as a source of energy, was the most important ingredient for maintaining adult longevity (Tsiropoulos 1980, Jácome et al. 1999). Like *T. nigricornis* and *T. bisetosa*, adults of *Anastrepha serpentina* (Wiedemann) could survive up to

5 d on diets lacking sucrose, such as intact fruits or bird feces (Jácome et al. 1999). The addition of proteins to a carbohydrate diet further increased longevity in *B. tryoni, A. serpentina* and *R. completa* (Drew 1987, Tsiropoulos 1980, Jácome et al. 1999). Contrary to *T. nigricornis* and *T. bisetosa, B. tryoni* and *B. cacuminatus* (Hering) survived longer, 4–5 months, on diets containing carbohydrates and proteins, such as autolyzed brewer's yeast or bacteria, compared to ca. 2 months with sucrose and water (Drew et al. 1983). Similar to *T. nigricornis* and *T. bisetosa*, no difference in longevity was detected between males and females of *A. serpentina* (Jácome et al. 1999). On the other hand, females of *R. completa* lived longer than males (Tsiropoulos 1980). But in *C. capitata, Bactrocera dorsalis* Hendel, and *B. cucurbitae* Coquillett, male longevity was greater than that of females and adult longevity was found to be influenced by temperature regimes (Vargas et al. 2000).

On protein hydrolysate diets, the longevity of ovipositing females of *T. nigricornis* and *T. bisetosa* was lower than that of females who had no access to oviposition sites. Egg production and egg laying were therefore exhausting stored nutrients and imposing a cost on survival. Similarly, in *C. capitata* females, egg production as well as mating were demonstrated to impose independent costs on survival (Chapman et al. 1998). Moreover, in *Anastrepha* spp., fertility and longevity seemed to be inversely related; species with higher fertility had lower longevity (Joachim-Bravo et al. 2003).

The fecundities of *T. nigricornis* (244 eggs) and *T. bisetosa* (158 eggs) on their natural hosts were close to that estimated for some of the fruit infesting species on sucrose plus yeast hydrolysate diets, *B. oleae* (200–250 eggs) (Christenson and Foote 1960), *Anastrepha obliqua* (Macquart) (274 eggs) (Joachim-Bravo et al. 2003), *A. serpentina* (164 eggs) (Jácome et al. 1999), slightly lower than *C. capitata* (300 eggs for wild flies), but much lower that *Anastrepha ludens* (Loew) (*ca.* 1400 eggs for reared flies) (Christenson and Foote 1960). The oviposition curve of *T. nigricornis* and *T. bisetosa* showed a high peak during the first week, then a smooth decline with time. Similarly, oviposition of different *Anastrepha* spp. was mainly concentrated in the first weeks of egg laying (Joachim-Bravo et al. 2003).

In non-choice experiments, *T. bisetosa* females could oviposit in the non-host heads of *E. farinosa*; however, *T. nigricornis* females could not successfully oviposit in the flower heads of wild sunflowers, the host of *T. bisetosa*, and their fecundity was greatly reduced. This was due to the physical features of wild sunflowers, which unlike the hosts of *T. nigricornis*, are covered by hard bracts and exude copious resins when pierced.

Unmated *T. nigricornis* and *T. bisetosa* females showed a drastically reduced fecundity (8–10 eggs) on their host plants. Similarly, virgin females of *B. oleae* matured eggs at the same time as mated females; however, their daily oviposition rates were lower than those of mated females (5–7 vs. 10–12 eggs/day). This suggested that mating offered a stimulus to oviposition and that high egg production was initiated once mating took place (Zervas 1983). Nevertheless, repeated matings did not stimulate oviposition of the apple maggot females. There was no significant difference in the number of eggs laid by females with frequent matings (395 eggs) and females with few matings (360 eggs) during the first two weeks. However, virgin females had a lower fecundity (ca. 100 eggs) compared to mated females (Nielson and McAllan 1965). Contrary to most tephritid species, no difference in egg

production was detected between virgin and non-virgin females of *C. capitata*. Mating was not necessary to induce egg production in this species, but it imposed a cost on the longevity of mated females (Chapman et al. 1998).

In conclusion, the sympatric and sibling species, *T. nigricornis* and *T. bisetosa* appear to have similar adult longevity, male maturation, and preoviposition periods. Females of both species required an extrinsic source of proteins to mature eggs. However, the fecundity of the specialist species *T. bisetosa* was significantly lower than that of the polyphagous *T. nigricornis*. Similarly, in the dacine flies, the fecundities of most oligophagous species were lower than those of the polyphagous species, and this was reflected in their survival strategies, which were much more r-selected than in the oligophagous species (Fletcher 1987). The higher fecundity of *T. nigricornis* probably facilitates the maximum use of its available hosts, which bloom for short periods (1 to 3 months) in the spring or in the fall. On the other hand, wild sunflowers, the main host of *T. bisetosa* which does not seem to diapause, bloom nearly throughout the year in southern California.

Apparently, these two sibling species, which show close morphological and ecological affinities (Knio et al. 1996a, b, 2001), have adopted different life history strategies. *Trupanea nigricornis* infests many plant species, has a wide incidence in the field, exists at higher densities, mainly in the spring and fall seasons, and has a higher fecundity than *T. bisetosa*, but it also suffers more mortality due to parasitism (Knio et al. 2007). On the other hand, the oligophagous species, *T. bisetosa*, exists at lower densities throughout the year on wild sunflower in southern California, it has a clumped distribution in the field, it suffers less pressure from natural enemies, but it has a lower fecundity than *T. nigricornis*. Thus, this high mortality due to parasitism is compensated by a high fecundity in *T. nigricornis*.

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