



Parasitism and Host Selection Behavior of *Trichopria columbiana* Ashmead and its Effect on Establishment and Dynamics of *Hydrellia* spp. Populations

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PURPOSE: This technical note examines and quantifies the effect of parasitism by *Trichopria columbiana* Ashmead (Hymenoptera: Diapriidae) on *Hydrellia* spp. emergence and also considers the influence and subsequent impact of these actions on population size in ponds at the Lewisville Aquatic Ecosystem Research Facility (LAERF), Lewisville, Texas. Field parasitism rates by *T. columbiana* were quantified and host selection behavior recorded. Host selection was examined by providing access to various immature life stages of *Hydrellia pakistanae* Deonier and quantifying oviposition behavior and wasp emergence.

BACKGROUND: *Hydrilla verticillata* (L.f.) Royle, in the family Hydrocharitaceae, is an introduced, submersed macrophyte that is a major problem in the United States (Sutton and Portier 1985). Its dense growth interferes with navigation, fishing, boating, and recreational activities (Baloch et al. 1980). Although herbicides are primarily used for control, biological control agents are becoming a viable option. In 1987, two species of leaf-mining flies in the genus *Hydrellia* (Diptera: Ephydriidae), *H. pakistanae* and *H. balciunasi* Bock, were released for management of hydrilla (Grodowitz et al. 1994).

Female *Hydrellia* spp. oviposit eggs on emergent hydrilla. Upon eclosion, the larvae (Figure 1) crawl along vegetation whereupon they enter and mine, on average, 9 to 12 hydrilla leaves (Baloch and Sana-Ullah 1974) during three instars. Upon completion of the third and final instar, larvae insert their respiratory spines into the hydrilla stem to pupate. The life cycle from egg to adult is completed in 17 to 31 days and is dependent on temperature (Baloch et al. 1976) and nitrogen content (Wheeler and Center 1996).

Numerous small and large-scale tank and pond studies have examined the impact of *Hydrellia* spp. feeding on hydrilla (Doyle et al. 2002, 2007; Grodowitz et al. 2004; and Owens et al. 2006); all have indicated significant reductions in hydrilla biomass, as well as tuber and turion production by sustained *Hydrellia* spp. herbivory. Similar effects have also been observed at several field locations (Grodowitz



Figure 1. *Hydrellia* spp. larva within a leaf of *Hydrilla verticillata*.

et al. 2003), although only minimal effects have been noted at other field locations. A number of ecological factors may explain variable impacts of *Hydrellia* spp. on hydrilla among field locations.

One factor may be related to the number of agents released at a particular location. Although establishment rates and range expansion across large geographical ranges have been noted for *H. pakistanae* (Center et al. 1997), sites with increased number and size of releases typically had higher local populations and subsequent impacts (Grodowitz et al. 2003). This has been aided to some extent by the development of innovative and efficient mass-rearing techniques using small ponds at both the LAERF and the U.S. Army Engineer Research and Development Center (ERDC) (Freedman et al. 2001, Harms et al. in review). These facilities have allowed production of large numbers of individuals with over 12,500,000 immatures released at 16 sites during 2005 alone. Despite the large number of flies released, some sites failed to establish, or established with subsequent low population increases.

Other factors may also be responsible for minimal population increases. These include increased predation by numerous generalist predators associated with hydrilla such as dragonflies and damselflies (Order: Odonata). Odonate nymphs are voracious feeders and are often found in large numbers associated with submerged vegetation. In addition, larval damselflies have been shown to feed on the obligate mining larvae of *Glyptotendipes* spp. (Diptera: Chironomidae) (Koperski 1998). Because of their close proximity to *Hydrellia* spp. immatures, larval predation may be an important population regulatory factor. In an unpublished study conducted at the LAERF, gut contents of damselflies (suborder: Zygoptera) and dragonflies (suborder: Anisoptera) were examined but no evidence was found of Odonate predation on the introduced larval *Hydrellia* spp. However, other studies using radioactive labeling techniques have identified odonate predation as an important factor (Cuda et al. 1997). Feeding by adult odonates on adult *Hydrellia* spp. has also been observed at field locations,¹ but no quantification has been attempted. More research is warranted.

Parasitism may be another possible reason for only minimal increases in *Hydrellia* spp. field populations. Parasitoids, by definition, are insects that develop inside another living organism and, through the course of development, kill the host organism. The genus *Trichopria* (Hymenoptera: Diapriidae), first described by Ashmead in 1893, wholly contains ectoparasitoids or endoparasitoids of dipteran larvae and pupae (Borror and DeLong 1981, DeBach 1964, Krombein et al. 1979). Because of the wide range of Dipteran families parasitized by *Trichopria* spp., its potential for use as a biological control agent against agricultural insect pests has been considered.

Trichopria columbiana is a generalist parasitoid wasp native to North America with widespread distribution (Merritt and Cummins 1996) and has been reported to parasitize at least two native *Hydrellia* spp. In addition, *T. columbiana* has been shown to parasitize *H. pakistanae* and *H. balciunasi* (Coon 2000). Female *T. columbiana* dives beneath the water surface to search for and parasitize immature life-stages (Figure 2) completing its life cycle from egg to adult in 19 to 24 days at 26°C and 100 percent RH (Coon 2000). After egg oviposition in the host puparium, *T. columbiana* undergoes three instars and a pupal stage within the submersed host. Upon emergence the adult wasp rises to the water surface enclosed in an air bubble (Coon 2000).

¹ Personal Observation. 2003. Michael J. Grodowitz, Research Entomologist, U.S. Army Engineer Research and Development Center, Vicksburg, MS.

Only minimal published information is available for *T. columbiana*, hence little is known about host selection, foraging mechanisms, and influence on host species' populations. Although its role in population dynamics of *H. pakistanae* has not been documented, it is apparently an important issue. Deonier (1971) states that "parasitic Hymenoptera undoubtedly exert considerable control on population densities of *Hydrellia*, especially in certain marginal habitats and when densities are very high." In a facility such as the LAERF where *Hydrellia* sp. densities can reach 6000 immatures/kg wet hydrilla (Figure 3), parasitism could play a major role in population regulation, and prevent existing populations from increasing to levels more detrimental to the hydrilla plant.



Figure 2. *Trichopria columbiana* parasitizing a *Hydrellia* spp. pupa.

The LAERF is located in Lewisville, Denton County, Texas, and is comprised of 53 earthen ponds ranging from 0.5-2.0 surface acres, averaging 1 m deep. Ponds were constructed in the 1950's and were used as game fish production ponds until 1985. Native macrophytes and macroinvertebrates are found in all ponds. Water to the ponds is gravity-fed from Lake Lewisville in Denton County, Texas (Smart et al. 1995). *Hydrellia pakistanae* and *H. balciunasi* were introduced to the LAERF accidentally in 1995 and since then various pond manipulation techniques have been used to increase their populations, including fertilization, water level adjustments, etc. (Harms et al., in review). In 1999, the flies inhabited just two ponds at the LAERF but by 2001 substantial fly populations were found in all ponds containing hydrilla. Currently, populations are large and several of the ponds are used for mass-rearing *Hydrellia* spp. for field release in the United States.

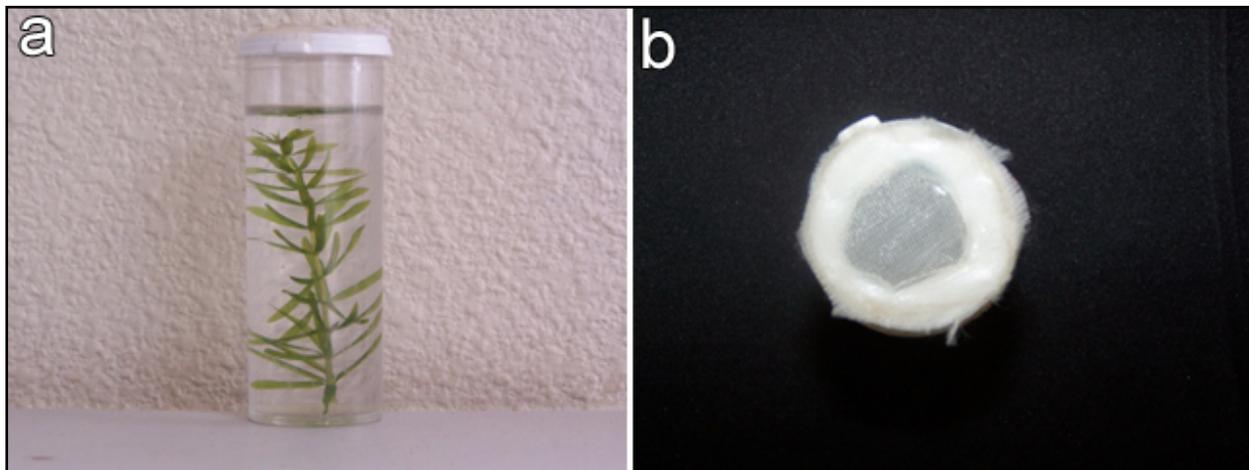


Figure 3. Plastic rearing vial containing 8- to 10-cm section of hydrilla (a). Ventilated lid of plastic rearing vial (b).

In an effort to more clearly understand the relationship between *Hydrellia* spp. population dynamics and *T. columbiana* parasitism, population numbers and parasitism were quantified for two years. Oviposition choice experiments using various combinations of *Hydrellia* spp. immatures were also conducted in order to more clearly define behavioral activities of *T. columbiana* based on time spent performing various activities during oviposition, as well as host life-stage preference for oviposition and associated wasp survival rates.

MATERIALS AND METHODS:

Hydrellia spp. Immatures per Kilogram at the LAERF. In 1999 and 2001, hydrilla stems were collected from rearing ponds at the LAERF, on average, every two weeks during the months of August through December (1999) and June through December (2001). Length and fresh weight were recorded and each stem was examined under a stereomicroscope. Microscopic examination was necessary to enumerate the number of *Hydrellia* spp. first, second, and third instar larvae, and pupae (collectively immatures) found on each hydrilla stem. Immatures per kilogram were determined per stem for each sample period.

Percent Parasitism at the LAERF.

Hydrellia spp. pupae were collected in 1999 and 2001 from rearing ponds at the LAERF, on average, every two weeks during the months of August through December (1999) and June through December (2001). Pupal collections coincided with hydrilla stem collections. The pupae were collected by sorting through hydrilla and clipping off stem areas containing a pupa. Each 8- to 10-cm section containing a single pupa was placed in a plastic rearing vial with a ventilated lid (Figure 3) and incubated in a growth chamber at 25°C with a photoperiod of 16 hr light:8 hr dark (Hot Pack Corp., Philadelphia, PA), (Figure 4). Vials contained sufficient water to cover stems, yet allowed room for an emerged adult to perch. Water was added as needed to maintain levels. All vials were checked daily and emergence of either an adult *Hydrellia* fly or the parasitic wasp, *T. columbiana* was recorded. Percent parasitism of *Hydrellia* spp., $[(\text{total } T. \text{ columbiana emergence} / \text{total pupae}) * 100]$, was calculated for each pond per sampling date and ponds were averaged together for each sampling date and month.



Figure 4. Growth chamber, containing rearing vials, used for incubation of *Hydrellia* spp. pupae.

Choice Experiments. Choice experiments were initiated to address three topics; 1) oviposition preference of *T. columbiana* to various *H. pakistanae* life stages, 2) percent survival of *T. columbiana* to adulthood when oviposited in a specific life stage of *H. pakistanae*, and 3) average percent time spent in each of four behavioral categories for all wasps observed and for wasps that oviposited. *Hydrellia pakistanae* were collected from an uncontaminated rearing colony and wasps collected from rearing ponds at the LAERF were identified as *T. columbiana* before use. Wasps were given the choice of two different *H. pakistanae* life stages and observed through a stereomicroscope in a Petri-dish for one hour. Life stages of *H. pakistanae* were designated as first, second, or third larval instars, and early, intermediate, or late pupae and were not extracted or detached from hydrilla during observations. The choice experiments were conducted 33 times. A stopwatch was used during the hour of observation to record the intervals of each behavioral category. Parasitized immatures were placed into a vial for incubation and wasp emergence was recorded. Incubation procedures were followed as listed in the percent parasitism section.

RESULTS AND DISCUSSION:

Hydrellia spp. Immatures per kg and Parasitism Rate at the LAERF. Average *Hydrellia* spp. immatures per kilogram at the LAERF followed a similar seasonal trend both years of the study (Figure 5). Average *Hydrellia* spp. immatures per kilogram increased 1.2-fold from August to October in 1999 and 2.4-fold in 2001 (Figure 5). Average *Hydrellia* spp. immatures per kilogram decreased 52 percent from October to December in 1999 and 66 percent in 2001 (Figure 5). *Hydrellia* spp. typically decline during winter months, corresponding to decreases in temperature and photoperiod and elimination of fly oviposition sites as hydrilla senesces thereby reducing biomass in the upper water column (Owens and Madsen 1998).

Mean percent parasitism followed a distinct seasonal trend both years of the study (Figure 5) and in 2001 a possible relationship between wasp and fly populations was noticed. In 1999 parasitism peaked in September and December at 12.7 and 12.0 percent parasitism, respectively. In 2001, percent parasitism increased 47-fold from August to a peak in December of nearly 30 percent parasitism. No trend was obvious in 1999; yet 2001 data combined with wasp developmental time indicated a possible density-dependent relationship between the parasitoid wasp and its host. Because *T. columbiana* develops from egg to adult in approximately 3-4 weeks, peaks in parasitism should follow peaks in *Hydrellia* spp. immature numbers by 3-4 weeks. For example, from October to November 2001 average percent parasitism sharply increased 3.1-fold, corresponding to October peaks of *Hydrellia* spp. immatures per kilogram. Following spikes in parasitism, immature numbers decreased 62 percent from October to November and continued to decrease into December. Since fly populations decreased at the onset of winter months when other factors (photoperiod, temperature, etc.) could also affect fly numbers, parasite-induced decreases could not be distinguished from seasonal declines.

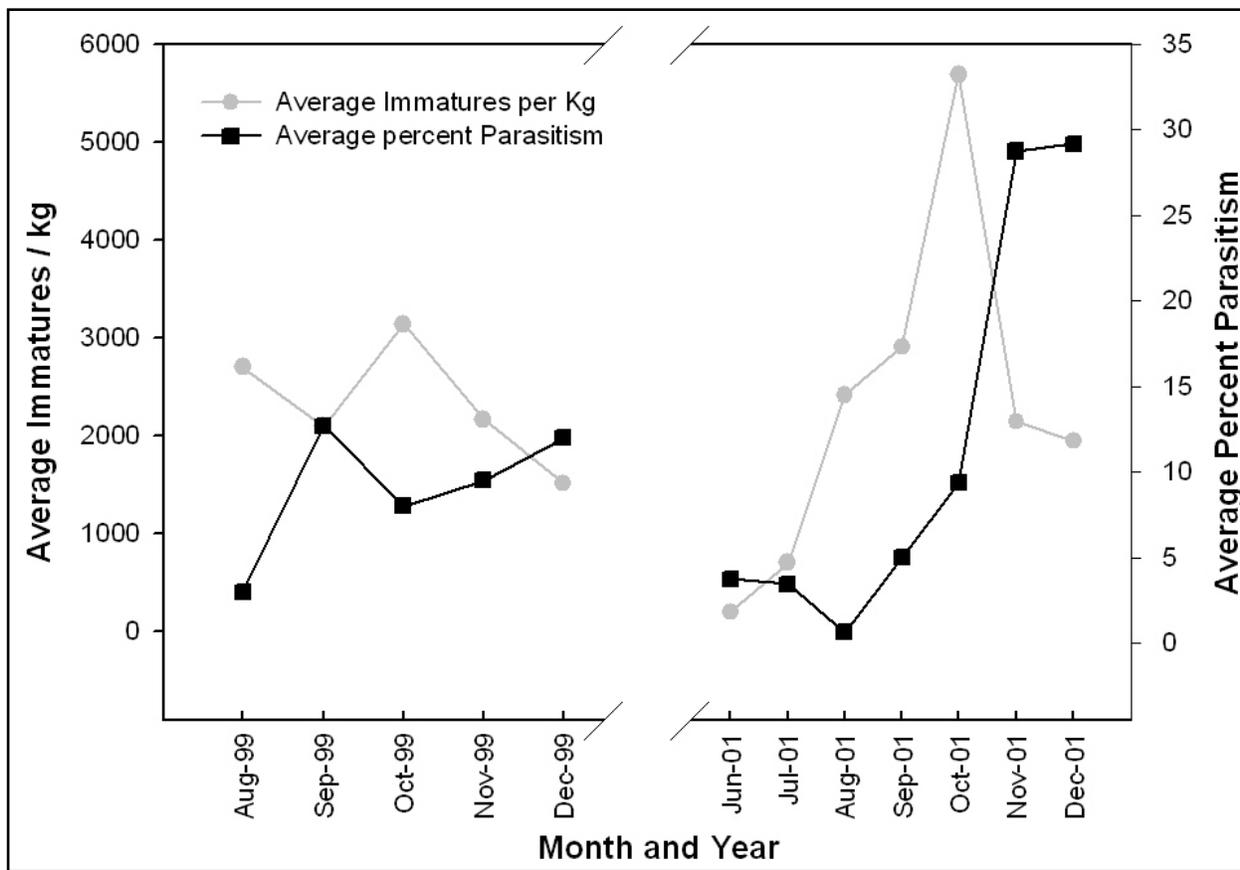


Figure 5. Average *Hydrellia* spp. immatures / kg and average percent parasitism by *T. columbiana* for 1999 and 2001.

From 1999 to 2001, *Hydrellia* spp. fly numbers increased despite a consecutive increase in parasitism levels of *T. columbiana*. In 1999 *Hydrellia* spp. fly populations peaked at 3,145 immatures per kilogram (Figure 5). In 2001, a 1.8-fold increase from the peak of 1999 was detected. *Hydrellia* spp. fly populations reached 5,693 immatures per kilogram in 2001 (Figure 5). From 1999 to 2001, *Trichopria columbiana* parasitism increased 2.3-fold from a peak of 12.7 percent parasitism in 1999 to nearly 29 percent parasitism in 2001 (Figure 5). Regardless of an increase in average percent parasitism from 1999 to 2001, fly populations appeared to be established and increasing without supplementation.

Choice Experiments: Oviposting Preference and Percent Survival. The most preferred oviposition life stages of *H. pakistanae*, late larvae and early to intermediate pupae, yielded the greatest percent survival of immature *T. columbiana* to adulthood (Figure 6). First instar larvae were not chosen by *T. columbiana* (Figure 6). A strong curvilinear relationship was found between percent oviposition and percent survival using a Pearson correlation analysis (Figure 7). This suggests that *T. columbiana* choose to oviposit in fly life stages that provide the highest percent survival for their young. Later fly immature stages would provide more food than early instars due to an increase in biomass. Late pupae, however, are so close to adulthood that their body structure may not be suitable for oviposition or feeding by immature wasps.

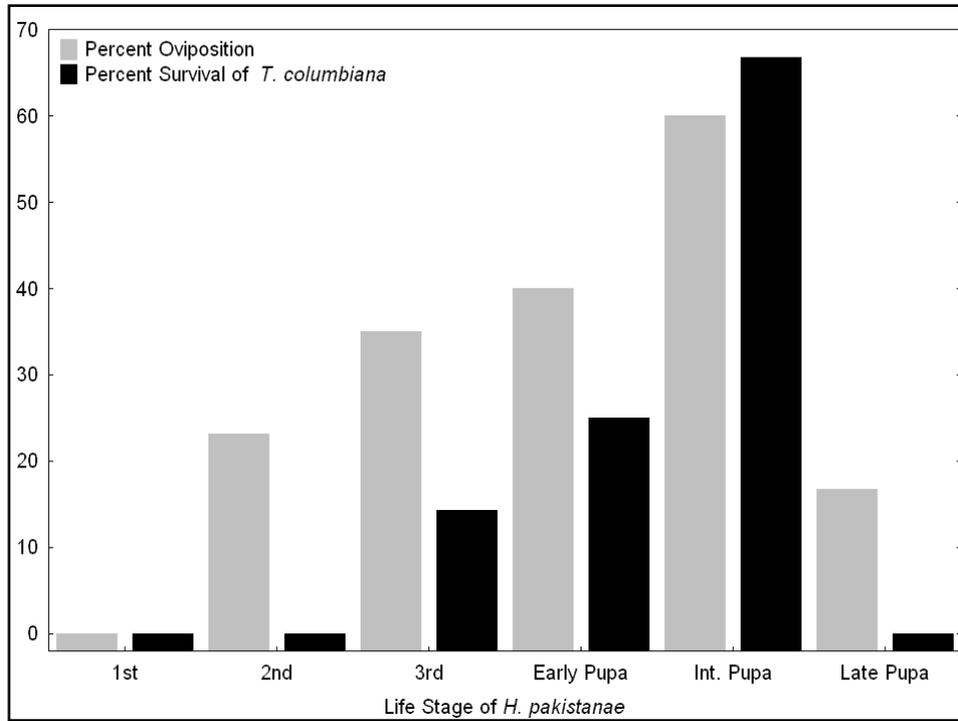


Figure 6. Percent oviposition and percent survival to adulthood of *T. columbiana* in life stages of *H. pakistanae*.

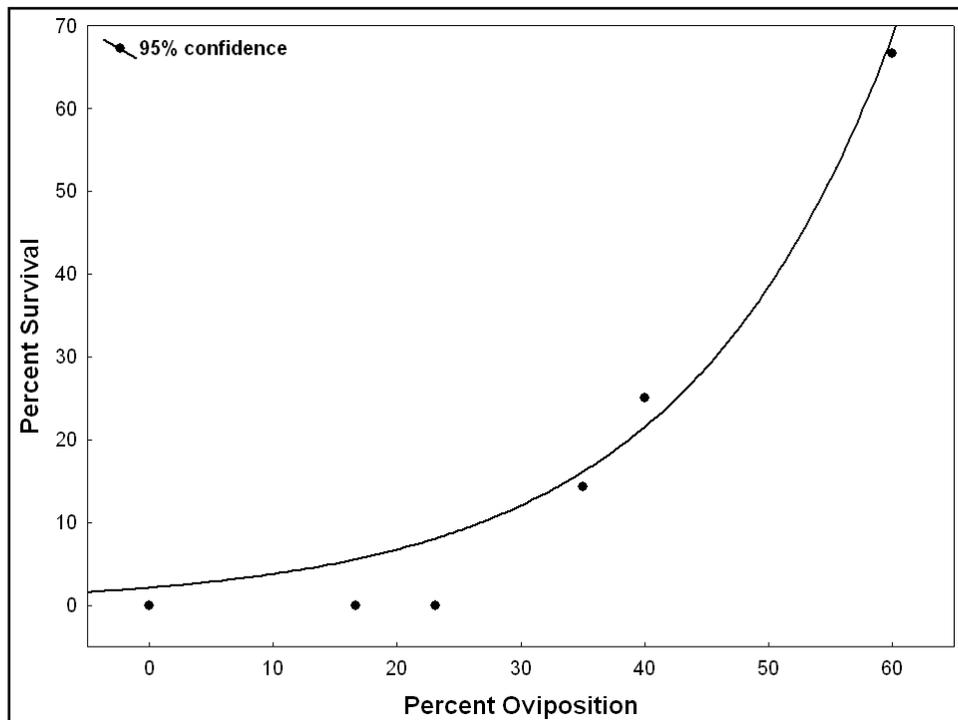


Figure 7. Pearson correlation analysis between percent oviposition and percent survival of *T. columbiana* in life stages of *H. pakistanae* ($p < 0.050$, $r = 0.89$).

Choice Experiments: Behavioral categories. After repeated observations of *T. columbiana* in the presence of hydrilla and *H. pakistanae* immatures, four general behavioral categories were described: searching, stem examination, ovipositing, and grooming/resting. When searching for a prospective host, wasps apparently drum their antennae against hydrilla leaves and stems while walking on hydrilla above and below the water's surface. Wasps examined stems with their ovipositor and/or mouthparts while remaining stationary. To oviposit, wasps pierced the immature host with its ovipositor in a stabbing motion. Grooming and resting were both accomplished while stationary and were often alternated. Wasps typically cleaned their antennae and legs. These four behaviors occurred in no specific order, but ovipositing was often followed by a grooming session.

On average, wasps spent the greatest percentage of time searching for prey to parasitize (58 percent) and the least examining stems (5 percent) (Figure 8). Grooming/resting were also a substantial component of the wasp's behavior (27 percent), yet *T. columbiana* only allocated 10 percent of time on average to ovipositing (Figure 8). Of the 33 wasps observed, only 15 oviposited. Due to the large number of wasps that did not oviposit during observation, time spent ovipositing could have been underestimated. For this reason the 15 wasps that oviposited were analyzed as a subgroup (known as ovipositing wasps) and average behavioral times were compared to the entire sample of wasps.

Percentage of time spent in each behavioral category was similar for both groups except for oviposition time (Figure 9). Ovipositing wasps spent the greatest percentage of time, on average, searching for prey to parasitize (53 percent) and the least examining stems (7 percent), while grooming/resting occupied 19 percent of the time (Figure 9). On average, 21 percent of time was spent ovipositing (Figure 9), a 2-fold increase over the entire sample of wasps. Regardless of ovipositing behavior, *T. columbiana* spent the most time searching for possible prey and the least time examining stems. By analyzing the ovipositing wasps, ovipositing was shown to be a more substantial component of *T. columbiana*'s behavior than originally observed.

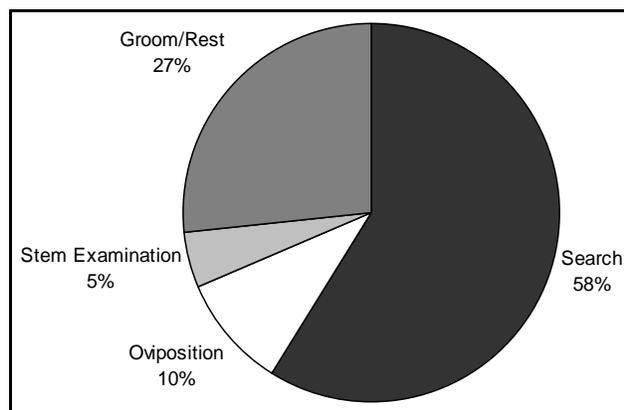


Figure 8. Average percent of time spent in each of four behavioral categories by all wasps observed.

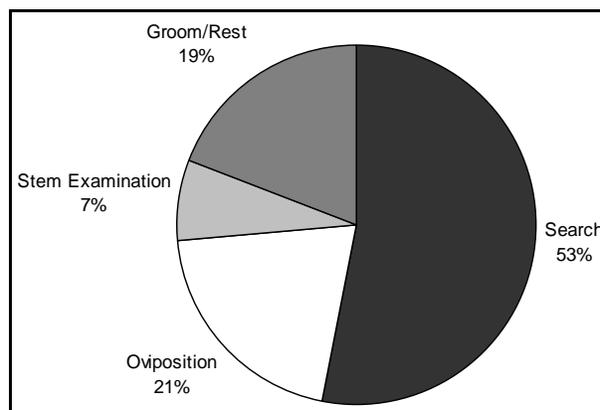


Figure 9. Average percent of time spent in each of four behavioral categories by the ovipositing subgroup of *T. columbiana*.

In conclusion, from 1999 to 2001, parasitism rates increased 2.3-fold at the LAERF, yet did not inhibit *Hydrellia* spp. populations from increasing 1.8-fold and establishing in four times as many ponds. Since decreases in *Hydrellia* spp. immatures / kg occurred at the onset of winter months and followed peaks in parasitism, a single factor could not be attributed to these declines. During choice

experiments, *T. columbiana* preferred to oviposit in late larvae and early to intermediate pupae. Consequently, life stages chosen most often for parasitism also yielded the highest percent survival of immature wasps to adulthood. The methods of host-stage selection are as yet unknown. Finally, four general behavioral categories were described for *T. columbiana*; ovipositing, searching, grooming/resting, and stem examination. Overall, wasps spent the greatest percentage of time searching for prey to parasitize and the least examining stems for oviposition sites. By examination of the ovipositing subgroup, it was shown that wasps allocated 21 percent of time to ovipositing.

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