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(Rhopalosiphum Nymphaeae) among Native and Invasive
Duckweeds (Lemnaceae)

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PREFERENCE AND PERFORMANCE OF THE WATER LILY APHID
(*RHOPALOSIPHUM NYMPHAEAE*) AMONG NATIVE AND INVASIVE
DUCKWEEDS (LEMNACEAE)

by

M. CAMERON STOREY

(Under the Direction of Alan Harvey)

ABSTRACT

Water lily aphids, *Rhopalosiphum nymphaeae*, are a polyphagous species of insect that feed on a variety of host plants including members of the Lemnaceae family. Many studies have focused on the relationship between herbivore preference and performance on different host plants, and as such the goal of this study was to determine if there is any relationship between host plant preference and performance of the water lily aphid on three different species of duckweed, including one invasive duckweed. Aphid preference was determined through a series of choice tests, which showed that the aphids preferred *Spirodela polyrhiza* over *Landoltia punctata* over *Lemna minor*. Water lily aphids also initially preferred the species they were reared on, even if it was not an overall preferred species, suggesting that familiarity plays a role in shaping host preference. To determine performance I measured offspring growth, reproduction and survival on all three species of duckweed. Aphids had the lowest performance levels on *Lemna minor* and the highest on *Landoltia punctata* and *Spirodela polyrhiza*. Aphids preferred and performed the best on the least nitrogen rich duckweed.

INDEX WORDS: Host-plant preference, Offspring performance, *Rhopalosiphum nymphaeae*, Duckweed, Maternal effects

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by

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BS, University of South Carolina - Aiken, 2004

A Thesis Submitted to the Graduate Faculty of Georgia Southern University in Partial

Fulfillment of the Requirements for the Degree

MASTER OF BIOLOGY

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CHAPTER 1

INTRODUCTION

The number of host species used by an insect population is an important component of niche breadth, and the use of several species may arise from preference differences among the insects (Singer 1983). Phytophagous insects can frequently be found in areas in which several suitable host species are located within close proximity (Via 1991). Some herbivores are locally monophagous but use different species in different parts of their range (Fox and Morrow 1981; Singer 1983). Fox and Morrow (1981) suggest that some insects are generalists whereas others are specialists because of the diversity in their local communities; therefore there is no *a priori* reason to predict that specialists will have greater efficiencies or competitive advantages over generalists. In contrast, the neural-constraints hypothesis predicts that generalist herbivores should make slower and poorer decisions than specialists when selecting plants, because generalists must discriminate and decide among stimuli from a wider variety of potential hosts (Bernays and Funk 1999). The brown Ambrosia aphid, *Uroleucon ambrosiae*, in its eastern region is very specialized and performs more efficiently at host-finding, host-selection, host-acceptance, host-sampling and host-settling than in its western region, where the aphid is more generalized which supports the neural-constraints hypothesis (Bernays and Funk 1999).

Mounting evidence suggests that individuals of polyphagous species do not use all available hosts equally (Fox and Morrow 1981; Via 1991). Host use in the field depends on both host availability and herbivore preference. The term ‘preference’ has been defined as the proportions of different food items in the diet relative to the

proportions of these same items that are available in the habitat (Hassell and Southwood 1978; Crawley 1984). Such preferences could reflect either environmental differences in the individual's familiarity with the range of available hosts or genetic, fitness-based differences (Via 1991).

An individual may be familiar with the host either via direct contact with the host plant itself or through indirect maternal effects. However, even if the insect has never come in contact with the host plant, studies have shown that populations can become acclimated to different hosts after certain periods of exposure (McCauley et al. 1990). Grain aphids, *Sitobion avenae*, that were collected from the field, were reared and tested on two different hosts. The grain aphids were able to survive on both hosts, as they had become acclimated to them (De Barro et al. 1995).

The Hopkins host-selection principle states that herbivores are more likely to feed on the plants they experienced as larvae (Hopkins 1917; Barron 2001). Herbivore host selection may also be influenced by plant chemical traces. The chemical legacy hypothesis states that insects are influenced by minute amounts of chemicals either in or on their bodies (Corbet 1985). The transfer of chemicals can occur at any time during the insect's development. Therefore, by feeding on or having chemical traces of a host plant on or in their body, the herbivore is then influenced to choose this familiar host.

Maternal effects are essentially the experiences of the mother and the outcome that her experience has on her offspring. In holometabolous insects female oviposition choices can affect both the growth and development of her offspring (Mousseau and Fox 1998). Singer et al. (1988) found that there was a relationship between maternal preference and offspring performance in terms of larval weight in the butterfly,

Euphydryas editha. Thorpe (1939) showed that there was an influence of the chemistry of the egg (maternal effect) on the behavior of the emerging insect. Experience can also lead to maternal effects on offspring quality (Mousseau and Dingle 1991). For example, in the noctuid moth *Heliothis virescens*, offspring of stressed mothers, which were fed a diet with secondary compounds were at a disadvantage in a stress environment relative to offspring of mothers fed on a plain artificial diet lacking the secondary compounds (Gould 1988).

Likewise, in hemimetabolous insects it seems likely that maternal effects can also influence the offspring. In aphids the experience of the mothers (maternal effects) on offspring performance seems particularly likely since they are a “telescoped” species (embryos of granddaughters are already developing within the embryonic daughters of a given female) (Dixon 1973; Via 1991; Dixon 1992). Aphid embryos are bathed in any ingested compound and this experience could potentially bias feeding preferences for certain host plants (Via 1991). Therefore, this suggests that maternal effects play a large role in shaping offspring preferences.

Often, however, insects exhibit host preferences that are not subject to change through experience. For example, Via (1991) tested pea aphid clones to determine if observed specialization could be significantly altered by prolonged experience on the alternate species. They found that regardless of exposure to the alternate host, there was no significant effect on the specialization of host performance of the two pea aphid clones. In these cases, it appears the preferences have a genetic basis usually thought to result from the differential fitness effects of the various potential hosts. In herbivorous insects, for example, higher nitrogen levels generally increase larval development and

growth rates (Tabashnik 1982; Prudic et al. 2005). As such, it would seem that herbivores may develop a preference for host plants that have high nitrogen quality. In fact, when presented with five different acceptable hosts, the beet armyworm, *Spodoptera exigua*, preferred and performed best on pigweed, which contained the highest concentrations of nitrogen when compared to the other hosts (Greenberg et al. 2001). Similarly, cotton aphid (*Aphis gossypii*) abundance was positively correlated with plant nitrogen content (Cisneros and Godfrey 2001). However, the butterfly, *Junonia coenia*, preferred to oviposit on host plants with high nitrogen levels even though there was no increase in larval performance on those hosts (Prudic et al. 2005). Nitrogen alone may not be the only factor influencing nutrient quality and therefore herbivore preference. Populations of the green peach aphid (*Myzus persicae*) were not significantly different among different nitrogen treatments, but were negatively correlated with the concentrations of amino acids found within different leaves (Jansson and Smilowitz 1986).

The term ‘performance’ is essentially a term used to describe some measure of fitness. There does not seem to be a set standard for measuring performance and instead a variety of characteristics have been used to indicate an insect’s performance. Some of the examples of these characteristics include measuring fecundity, larval weight, larval growth, larval and pupal development time and survivorship (Singer et al. 1988; Via 1991; Bowers et al. 1992; Cronin et al. 2001; Solarz and Newman 2001; Jallow and Zalucki 2003). For this study performance is defined as a measure of the intrinsic rate of increase (r_{\max}). The (r_{\max}) values represent the combination of development, growth, reproduction and survivorship. Essentially the (r_{\max}) values for performance can be thought of as a direct measurement of fitness.

Singer et al. (1988) suggests that different populations of insects will prefer and perform best on their own host species when compared with hosts from other populations. Any tendency for this to occur, suggests that there is a positive correlation between preference and performance. This correlation is an important factor in the maintenance of diet breadth. In other words, preferences should track performance. Such as with the positive relationship between preference and performance found in the butterfly, *Euphydryas phaeton*, which oviposits preferentially and performs best on its native host, turtlehead (*Chelone glabra*) (Bowers et al. 1992).

One adaptation that is expected to arise where plant genotypes differ significantly in their suitability for herbivore growth, reproduction and survivorship is a preference. Female herbivorous insects should oviposit preferentially on plants in which fitness is optimized (Cronin et al. 2001; Shiojiri and Takabayashi 2003). Other reasons that can result in a positive relationship between preference and performance on novel hosts are as a host becomes more acceptable, the acclimated organism may feed more than if it was not acclimated. The use of detoxification enzymes, which play a role in metabolizing toxic compounds in many herbivores, could also become more acceptable in the novel hosts (Feyereisen 1999). Preference and performance can also provide benefits similar to genetic specialization, where there is a focused ability to find certain hosts and evade their defenses (Agrawal et al. 2002).

However, female preference may not always lead to increased fitness in herbivores. Quite frequently herbivorous insects show a preference for the host which is not best for their offspring (Mayhew 2001). For example, Australia female cotton bollworms (*Helicoverpa armigera* (Hübner)) prefer to oviposit their eggs on maize (*Zea*

mays) even though the eggs typically have lower survival and growth rates (Jallow and Zalucki 2003).

Females may be ovipositing on these fitness-reducing host plants for a multitude of reasons. Some of the reasons could include competition, predation, disease, parasitism, or that the mother made a mistake and chose the wrong host plant (Thompson 1988; Mousseau and Fox 1998; Digweed 2006). In some cases, adult females oviposit on the host plant that is most preferred by the adult. *Chromatomyia nigra* were found to oviposit on plants where they feed, and they feed on host plants that enhance adult rather than larval performance (Scheirs et al. 2000; Mayhew 2001). This implies that by enhancing their own survival and fecundity, they may be able to have more offspring (Scheirs et al. 2000).

Most of the studies listed previously have been laboratory studies that have not incorporated those factors, besides host nutritional quality, that can directly impact offspring fitness. Experiments conducted in the field do encounter these biotic and abiotic factors which do influence fitness. When tested in the field the stem-galling fly, *Eurosta solidaginis*, demonstrates a weak correlation between host preference and offspring performance (Cronin et al. 2001). Also the birch-leaf mining sawfly (*Profenusa thomsoni*) has a very weak relationship between oviposition preference and larval performance when tested in the field. Females have been shown to oviposit their eggs to avoid competition and also to reduce larval exposure to predators or parasitoids (Digweed 2006). Therefore, evolutionary theory would predict that host preferences would evolve to maximize overall fitness, not just performance.

The relationship between host preference and offspring performance may also depend on the developmental pathways of a species. Holometabolous insects, which undergo a complete metamorphosis demonstrate different features as larvae and then later as adults. Adults are winged and able to search great distances for acceptable hosts, whereas the young usually have limited mobility and are not able to move long distances during their first few instars (Prudic et al. 2005). Also the hosts that provide high fitness levels for the adults may be different than those of the larvae because they have such different body forms and need different requirements. Hemimetabolous insects may also be at a disadvantage because there is competition between the parent and offspring sharing the same host plant. However, in some hemimetabolous insects, such as aphids, the plant from which an individual aphid originated is an important determinant of whether or not colonization of a new plant will be successful (Via 1991).

The role of invasive species

How are new host plants incorporated into the diet of an herbivore? At least two criteria must be met: (1) the herbivore must be able to recognize the novel plant as a food source and (2) the herbivore must be able to survive on it (Bowers et al. 1992). In crop systems, herbivores incorporate novel crop species into their diet if it is similar or closely related to the natural host plant. For example, the butterfly *Colias philodice eriphyle* was able to incorporate alfalfa into its diet because of the similarities to the native legume host (Tabashnik 1983).

Host selection shifts in natural plant and insect populations have rarely been studied (Bowers et al. 1992). Host shifts can occur for a number of reasons, including chemical similarities, geographic proximity, physical and temporal suitability, and

perhaps even the introduction of a novel or introduced species (Berenbaum and Zangerl 1991). Introduced or alien plants are plant taxa whose presence in a given area is due to intentional or accidental introduction (Richardson et al. 2000). It is proposed that at least 50,000 alien species have been introduced into the United States (Pimentel et al. 2000). A number of biotic and abiotic consequences have been recognized with the introduction of these alien species. Some of these consequences include negative impacts within ecosystems as well as economic impacts due to problems caused by the introduced species. However, little attention has focused on the impact that introduced species are having on the replacement of native plants in terms of insect herbivore palatability (Tallamy 2004). Native plants are palatable to a wide array of both specialist and generalist herbivores, but introduced plant species are predicted to be either partially or entirely unpalatable to most native phytophagous insects (Tallamy 2004).

Introduced species can displace native plants and thereby alter the availability of host plants for herbivores (Solarz and Newman 2001). However, even if natives are not displaced a successful invader can offer another food option for herbivores. On the other hand, introduced species can only be considered a food choice if the herbivore can recognize and feed off it. Bowers et al (1992) suggested that an increase in invasive plant availability might influence herbivore preference. Some examples of herbivores that have increased their host use to incorporate an introduced plant species include the butterfly *Euphydryas phaeton* and the milfoil weevil *Euhrychiopsis lecontei* Dietz (Bowers et al. 1992; Solarz and Newman 2001). In fact the milfoil weevil, *Euhrychiopsis lecontei* Dietz, has recently been shown to demonstrate a preference for the introduced Eurasian

watermilfoil (*Myriophyllum spicatum* L.) over its native ancestral host the northern watermilfoil (*Myriophyllum sibiricum* Komarov) (Solarz and Newman 2001).

Although a successful plant invader would seem to provide additional host choices to herbivores, this is often not the case. In fact, the escape-from-enemies hypothesis suggests that the success of an invader depends on reduced herbivory by locals. This hypothesis suggests that plants are suppressed in their native range by natural enemies and that escape from these enemies enables alien populations to grow explosively in communities into which they are introduced (Callaway and Aschehoug 2000; Maron and Vilà 2001; Wolfe 2002; Dietz et al. 2004; Vilà et al. 2005). This is illustrated by Wolfe's (2002) comparison of *Silene latifolia* which in its native range in Europe suffered more enemy damage than in its introduced range of North America.

Aphids as a model organism

Aphids are a perennial source of frustration to farmers and gardeners as they are phloem feeders and major vectors of plant viruses. Aphids are important pests of agricultural and horticultural crops worldwide, which continue to have a severe economic impact year after year (Center et al. 2002; Powell et al. 2006). Due to this direct economic impact, a greater need has arisen to understand aphid settling and reproduction on different plant species. There are also several biological characteristics that make aphids a widely studied model organism. First aphids are parthenogenetic; they are capable of reproducing without males. These asexual females also produce live offspring. Second, aphids have the ability to start development at ovulation, which shortens the time between birth and reproductive maturity. Third, many aphid species have evolved alary polyphenism; some aphid species produce both winged (alate) and wingless (apterous)

adults (Dixon 1973; Powell et al. 2006). These combined characteristics have given aphids a further fitness advantage, and also make them a desirable study species.

Objectives of study

For this study the water lily aphid (*Rhopalosiphum nymphaeae*) will be used to identify the factors involved in the relationship of host plant preference and offspring performance. The water lily aphid is an excellent species for this study. First, *R. nymphaeae* is a polyphagous species of aphid that feeds on a broad range of aquatic and semi-aquatic plants, including water lilies (*Lilium* spp.), American lotus (*Nelumbo lutea*), arrowheads (*Sagittaria* spp.), bladderworts (*Urticularia* spp.), cattails (*Typha* spp.), pickerelweed (*Pontederia cordata*), pondweeds (*Potamogeton* spp.), rice (*Oryza sativa*), spatterdock (*Nuphar luteum*), water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), water milfoils (*Myriophyllum* spp.), and duckweeds (Lemnaceae), as well as cultivated fruit trees including pear and peach (Center et al. 2002). Water lily aphids are heteroecious; they migrate from aquatic habitats to fruit trees in late fall. Females lay eggs on the fruit trees as part of the over-wintering stage, then subsequent generations migrate to aquatic plants in mid to late summer.

Second, as *R. nymphaeae* has a worldwide distribution, findings from this experiment can be compared to other aphids in various locations. Third, water lily aphids are easy to rear in the laboratory (Hance et al. 1994), which is important when performing entire experiments in a laboratory setting, such as in this study. Finally, aphids grow and reproduce quickly. While on their aquatic hosts, the aphids are ovoviviparous and give birth to live young. Water lily aphids are capable of reproducing one offspring nearly every six hours (Dixon 1973). Water lily aphids progress through

five instars. The development period from birth to the adult stage (fifth instar) ranges from 7 to 10 days depending on the temperature, with an optimal range from 21 to 27°C (Hance et al. 1994). This last characteristic is critical to my study because it allowed me to see treatment effects on life history traits in a short period of time.

Water lily aphids can readily walk on the water's surface in search of suitable hosts and use their stylet to probe plants before selecting a host (Scotland 1940). After colonizing aquatic sites, aphids reproduce rapidly, often virtually blanketing the hydrophytes present. The water lily aphid is extremely destructive and can transmit at least five plant viruses (Dixon 1973; Ballou et al. 1986; Center et al. 2002)

Preferences among aphids have widely been noted in literature, including the pea aphid, which demonstrates pronounced preferences among crop species (Via 1991). The host plant species used in this study was duckweed (Lemnaceae). Duckweeds are tiny free-floating vascular plants with world-wide distribution. There are five common genera, *Spirodela*, *Lemna*, *Landoltia*, *Wolffia* and *Wolffiella* and about 37 species (Rusoff et al. 1980; Landolt 1986; Landolt and Kandeler 1987; Les et al. 1997; Les and Crawford 1999). Duckweeds reproduce by vegetative reproduction and are characterized by rapid clonal growth. The plants cluster in colonies and form green blankets or a type of mat on the surface of the water (Hillman 1961). Duckweed was chosen because of its world-wide distribution and rapid growth rates to keep up with the rapid rates of the aphids.

I used three species of duckweed in this study: *Landoltia punctata*, *Lemna minor* and *Spirodela polyrhiza*. Scotland (1940) noted that *R. nymphaeae* have been found on all three of these species of duckweed. *Landoltia punctata* is an introduced species. *Landoltia punctata* is native to Asia and was first recorded in the United States in the

1930's, and is now found throughout the southeastern United States (Les et al. 1997), *Spirodela polyrhiza* and *Lemna minor* are native to the region (Landolt 1986; Landolt and Kandeler 1987). All three species reproduce almost entirely through vegetative means. Asexual reproduction occurs quickly; frond numbers can double every twenty-four hours (Landolt 1986; Landolt and Kandeler 1987). *Lemna minor* is one of the smaller species of duckweed, with an average frond length of 3 – 4mm; fronds are round with a single root. *Landoltia punctata* fronds are slightly larger than *L. minor*, slightly more elongated in frond shape and usually have multiple roots. *Spirodela polyrhiza* is the largest of the duckweeds, ranging in size up to 8mm with multiple roots per frond and often with a single red dot located on top of the fronds near the node. *Spirodela polyrhiza* and *L. punctata* are also easy to distinguish from *L. minor* as they are purplish-red underneath the fronds (Landolt 1986; Landolt and Kandeler 1987).

The overall goal of this study was to determine aphid preference and performance among species of native and invasive duckweeds. Specifically, I addressed the following questions 1) Do water lily aphids show preferences among duckweed species? I hypothesize that *R. nymphaeae* will demonstrate a preference since much of the literature alludes to polyphagous insects exhibiting a preference, such as with the pea aphid (*Acyrtosiphon pisum*) that is polyphagous but prefers alfalfa over other crop hosts (Via 1991). I also hypothesize that the aphids will prefer the native duckweed over the invasive. They would not prefer the invasive species because they are not familiar with it and not adapted to it, much like *Euphydryas phaeton* which preferred the native host plant. 2) If *R. nymphaeae* do show a preference, are these preferences influenced by

previous experience? Because aphids are hemimetabolous insects, and are born live, I predict that maternal effects will play a large part in affecting offspring preference.

3) Does preference track performance; meaning is there a positive relationship between host-plant preference and offspring performance? Many of the studies have shown that holometabolous insects have either a weak or negative relationship between preference and performance, such as the Australian cotton bollworm ovipositing on maize (Jallow and Zalucki 2001). However, particular aphids, like the pea aphid have shown a positive correlation between host plant preference and offspring performance. Thus, I hypothesize that water lily aphids will have a positive relationship between preference and performance. 4) Do preferences reflect host quality? If the aphids demonstrate a preference it would be interesting to show how and/or if the preference is linked to host quality. I hypothesize that the preferred host species will also have the highest quality in terms of nutrients. Since several insects, including the cotton aphid are linked to plants with higher nitrogen content, it seems likely that water lily aphids would also prefer a nitrogen rich host plant. 5) Does nutrition of the duckweeds affect offspring performance among the aphids? I hypothesize, that if the aphids prefer a poorer quality host species, then their performance will be less than that of those aphids preferring a higher quality host especially in terms of offspring survival, growth and reproduction.

CHAPTER 2

METHODS

Duckweed rearing methods

Duckweed was collected from two local bodies of water (GPS coordinates, site 1: 32°27.807N, 81°48.546W; site 2: 32°23.642N, 81°46.412W; Figure1). The collection sites were within a 20 mile radius of Georgia Southern University, Statesboro, Georgia, USA campus. Collections were sorted in the laboratory. *Landoltia punctata* was by far the most widespread and abundant duckweed species collected in the area.

Each species of duckweed was cultured separately in 11.4L Aero™ plastic, black tubs (30cm long x 28cm wide). I initially filled the tubs with 8L tap water and 20mL of Dyna-Gro™ (7-9-5) liquid plant food. Due to evaporation, I topped off the tubs weekly with distilled water. Approximately every two weeks (or sooner if the duckweed looked unhealthy), I started fresh cultures using a small amount (approximately the size of the palm of your hand) of duckweed from the older tubs. The tubs were placed underneath two standard Sylvania fluorescent lights (one was a 40W warm light lamp, which is used for plants and aquariums and one was a 40W cool light lamp, which is standard for use in basement settings). These temperature lamps were used to best maintain the optimal temperature range for the water lily aphids, which is between 21 – 27°C. The light regime was a 12:12 (L/D) hour photoperiod (previous personal observation had shown aphids did well with these lighting conditions).

Aphid rearing methods

Water lily aphids used in this study were from a laboratory colony. The colony was originally founded from aphids collected from a *Spirodela polyrhiza* duckweed

sample purchased from a water garden nursery in Florida. The duckweed sample was originally purchased in the spring of 2005. Initially five to ten aphids were placed into each duckweed tub and maintained along with the duckweed. Because I was interested in the influence of maternal effects on aphid preference and performance, I needed to ensure that aphids in a tub only had experience with the duckweed species in that tub. So, I covered the tubs with thin transparent plastic Plexiglas sheets (31 cm long x 29 cm wide) to prevent the aphids from flying to different tubs.

Aphid mobility

Any preference experiment requires that the organism be mobile enough to encounter each alternative. To assess the mobility of *Rhopalosiphum nymphaeae*, I monitored the location of individual aphids over several days. I filled six 750 mL plastic blue cups with 500 mL of distilled water and 5 mL of full-strength Dyna-Gro™ (7-9-5) liquid plant food. Two containers held *Lemna minor*, two contained *Landoltia punctata*, and two contained *Spirodela polyrhiza*. Because duckweed floats freely on water, any movement in a container caused the fronds to be jostled, changing the position of feeding aphids. To minimize frond movement, I placed thin white plastic mesh sheets, cut to size (8 cm in diameter), inside the cup before the duckweed was added. The plastic mesh was held in place by a jumbo paper clip on the outside of the cup. The paper clip also served as a reference point for aphid movement. When duckweed was added to the cups, the fronds rested on or floated just above the mesh while the roots passed through the mesh, effectively holding the duckweed in place. Five adult aphids from each of the three species of duckweed were haphazardly placed in each of the six containers. In total there were ten aphids on *Lemna minor*, ten aphids on *Landoltia punctata*, and ten aphids on

Spirodela polyrhiza, with a total of ten readings per aphid. Digital photos were taken using a Sony Cyber-shot™ digital camera with a 3x optical zoom. Photos were taken at twelve hour intervals (approximately 7am and 7pm EDT) for five days. I checked the position of each aphid through the sequence of photos to determine whether each aphid changed position. I cannot be sure of which aphid was which because they were not marked for this study, however I am sure that the aphids and any of their subsequent offspring did move because through the photos, a majority of the aphids were never on the same duckweed.

Preferences of aphids among duckweeds

To determine the host preference of aphids among duckweed species, I reared separate colonies of aphids on each species of duckweed for at least four generations, which ensured that the aphids had only experienced one species of duckweed. I haphazardly selected three adult aphids (determined by eye based on appearance and size) from each species of duckweed (Figure 2). Each aphid was placed in a 35mm black film canister that contained 25mL of standard nutrient solution (i.e., a mixture of 10mL of Dyna-Gro™ (7-9-5) liquid plant food combined with 10L of distilled water) and approximately equal parts (50:50, judged by eye) of two duckweed species (either *Lemna minor* and *Landoltia punctata*, *L. minor* and *Spirodela polyrhiza*, or *L. punctata* and *S. polyrhiza*). Figure 2 shows the experimental design. Each film canister was only filled 90%, in order to leave room for the duckweed to grow. The film canisters were then covered with Falcon® 35 x 10mm Petri dishes to prevent the aphids from escaping. Canisters were haphazardly placed underneath the grow lights in the laboratory. The light regime was a 12:12 (L/D) hour photoperiod.

I recorded the location, i.e. which duckweed species the aphid was on, for each aphid after one hour, and then every twelve hours for the next three days. During the mobility study I observed that some aphids would move as quickly as thirty seconds, so one hour was a precautionary measure to ensure that all aphids had time to move. I tested a total of 153 aphids, with 17 aphids per treatment. Aphids that were not located on either species of duckweed at the time of data collection were excluded from statistical analysis.

Statistical analysis of preferences

To assess the overall preference of the aphids, I used the third reading (i.e., twenty-five hours after the trial started). The third reading was used because mobility tests had shown that twenty-five hours was enough time for aphids to move around. I used a Chi-square test to see if aphids were significantly more likely to be found on one test species.

However, to assess the influence of previous experience on these preferences, I used the first reading (one hour after the trial started) because that was the reading at which the aphid would have had the least experience with the other species. I used a Chi-square test to see whether aphids were likely to choose the more preferred species after only one hour as they were after twenty-five hours.

Performance of aphids on different duckweed species

To determine whether the three duckweed species affected aphid fitness and to determine if performance was positively correlated with aphid host preference, I used a set up similar to the preference experiment, except that each container held only one duckweed species. Aphids were tested on each duckweed species, for a total of nine treatment combinations (Figure 3). I haphazardly placed selected first-day first instar

aphids singly into testing containers with a single duckweed species. Again the canisters were only filled 90% of the way, to allow room for the duckweed to continue growing during the experiment. Canisters were haphazardly placed underneath the fluorescent lights in the laboratory. Because many components of fitness in insects are known to be influenced by temperature, and because I had no way to directly control the temperatures in the lab where these experiments were conducted, I recorded temperature every hour for the duration of the experiment using a HOBO temperature monitor.

I checked each canister daily and recorded any instances of mortality, molting, or reproduction. Molting was determined by the presence of exuviae. All offspring were removed from the containers each day. I tested a total of 180 aphids, with 20 replicates of each treatment combination. I calculated for each aphid the number of pre-reproductive days, the number of reproductive days, the number of post-reproductive days, the number of offspring, and the total lifespan.

Statistical analysis of performance

All of the performance experiments were analyzed using a two-way analysis of variance (ANOVA) with interaction between the rearing duckweed species and the test duckweed species. In all performance experiments the two main factors were rearing duckweed species and test duckweed species. Also pair-wise comparisons of all performance experiments were compared using a Tukey-Kramer HSD. The lengths (in days) of the pre-reproductive, reproductive, and post-reproductive periods were compared. The number of offspring produced was also analyzed. Individual fertility tables were constructed for each reproductive aphid. The fertility tables were constructed by calculating the number of offspring (R_0) and then calculating the number of offspring

times the number of the day on which the offspring were produced ($\sum l_x m_x$). From that the generation times (T_c) and intrinsic rate of increase (r_{max}) were calculated for each reproductive aphid. Generation time was found using the following formula: $(T_c) = (\sum l_x m_x) / (R_0)$. The intrinsic rate of increase (r_{max}) was calculated using this formula: $(r_{max}) = (\text{natural log } R_0) / (T_c)$ (Mondor and Roitberg 2003).

I analyzed the effects of rearing species and tested species on aphid generation times (T_c) using a two-way ANOVA. A Tukey-Kramer HSD test was used to test for pair-wise comparisons. The intrinsic rate of increase (r_{max}) was also analyzed using a two-way ANOVA with a Tukey-Kramer HSD for pair-wise comparisons.

Duckweed nutrient analysis

To determine if preference and performance are influenced by host quality, I determined the nutrient composition, i.e. nitrogen, carbon and hydrogen content for each species of duckweed. Nitrogen content is considered a key factor in plant-herbivore interactions (Perez-Harguindeguy et al. 2003), and as such was the main nutrient focused on in this study. Samples were taken from at least three different rearing tubs, in order to test a broader range of the duckweeds. I haphazardly collected samples from fresh colonies, none that were older than 10 days. For the duration of this study, none of the duckweed colonies used ever reached over 20 days, so 10 days was the halfway point. I oven-dried samples of each species at 50°C and then ground them into a fine mesh. Seven 2mg samples were then sent to the University of Georgia's Chemical Analysis Laboratory. Analysis for nitrogen content was done using a Perkin Elmer 2400 Carbon Hydrogen Nitrogen Analyzer.

Statistical analysis of duckweed nutrients

To compare nutrient levels across species, I ran an ANOVA for nitrogen with a Tukey-Kramer HSD test for pair-wise comparisons. For all experiments, tests for normality and homogeneity of variance were performed and all analyses were done using JMP IN 5.1.

CHAPTER 3

RESULTS

Mobility

Overall, aphids were very likely to move between the 12 hour readings. The pictures showed that aphids were mobile on each duckweed species, but most active on the *L. punctata* with those aphids having a 90% chance of having a new position. Aphids on *L. minor* and *S. polyrhiza* all had an 80% chance of being in a new position between readings (Figure 4).

Preference

Aphids preferred *S. polyrhiza* over *L. punctata* ($\chi^2 = 14.225$, $P < 0.001$) and *L. minor* ($\chi^2 = 10.939$, $P < 0.001$), and *L. punctata* was preferred over *L. minor* ($\chi^2 = 6.125$, $P < 0.05$; Figure 5A-5C, Table 1). For the first readings, aphids favored the species they were reared on (whenever possible), even if it was overall the less preferred species of the two options (Table 2). Aphids that were reared on the more preferred of the two species and then chose the preferred species, does not offer any insight into host preference selection. Thus the choice could be due to maternal effects or preference. Therefore the aphids that were reared on the less preferred species, offer the greatest idea of the mechanism behind host preference. If rearing does not matter, then the initial preference should match the overall (day three) preference. Overall, aphids chose the preferred species 79% of the time and the less preferred species 21% of the time. For the first reading, aphids reared on less-preferred species chose that species 71% of the time, the preferred species only 29%.

Performance

The length of the pre-reproductive period was significantly different for the duckweed species on which the aphid was reared ($F_{2,78} = 2.83$, $P = 0.0087$; Figure 6). Aphids reared on *L. minor* had significantly longer pre-reproductive days than either *L. punctata* or *S. polyrhiza* ($q = 6.08$ Tukey-Kramer HSD). The duckweed species that the aphid was tested upon was significantly different for the length of the reproductive period ($F_{2,78} = 2.79$, $P = 0.0081$; Figure 7), with again *L. minor* being significantly different from both *L. punctata* and *S. polyrhiza* ($q = 2.63$ Tukey-Kramer HSD). However, the length of the post-reproductive period did not differ significantly for aphids, in terms of rearing or test duckweed species ($q = 1.78$ Tukey-Kramer HSD; Figure 8).

I measured the average number of offspring produced by each adult aphid over the duration of the study. Fecundity was affected by the species of duckweed on which the aphid was tested ($F_{2,78} = 3.30$, $P = 0.02$; Figure 9). Aphids tested on *L. minor* had a significantly smaller fecundity than aphids tested on either *L. punctata* or *S. polyrhiza* ($q = 2.39$ Tukey-Kramer HSD). *Landoltia punctata* reared aphids produced an average of over 12 offspring when tested on *L. punctata* as likewise with *S. polyrhiza* aphids producing an average of 14.4 aphids. *Lemna minor* aphids did not reproduce well on any species tested, with an average of only one offspring per individual.

In general, aphid development was more strongly influenced by the duckweed species on which its parent was reared than by the duckweed species on which it was tested. Generation times of the aphids were significantly affected by the duckweed species on which they were tested ($F_{2,78} = 1.576$, $P = 0.0378$; Table 3) with *L. minor* tested aphids being significantly different from both *L. punctata* and *S. polyrhiza* ($q =$

6.44 Tukey-Kramer HSD). Aphids tested on *S. polyrhiza* had the longest generation times (mean \pm 10.46), and aphids tested on *L. minor* had the shortest generation times (mean \pm 6.29). Not surprisingly, the intrinsic rates of increase of the aphids were significantly affected by the duckweed species on which the aphid was tested ($F_{2,78} = 6.775$, $P < 0.0001$; Table 4). Only aphids tested on *L. minor* were significantly lower from aphids tested on *L. punctata* and *S. polyrhiza* ($q = 0.04$ Tukey-Kramer HSD). Aphids reared and tested on *L. minor* had the lowest values for intrinsic rates of increase, showing their overall poor fitness level. By graphing aphid preference (percentages of preference data) and offspring performance (r_{\max} values) it shows the relationship of these two factors among the three species of duckweed. Because *S. polyrhiza* was the most preferred duckweed species it is higher on the graph than *L. punctata*, despite the fact that there was no significant difference in the intrinsic rates of increase between these two species (Figure 11).

Nutrient analysis

Nitrogen concentrations varied significantly among the three species of duckweed. *Lemna minor* contained a significantly higher proportion of nitrogen ($F_{2,18} = 4.14$, $P = 0.03$; Figure 10) than either *L. punctata* and *S. polyrhiza* ($q = 2.55$ Tukey-Kramer HSD).

CHAPTER 4

DISCUSSION

The overall goal of this thesis was to determine the host-plant preference and offspring performance of *Rhopalosiphum nymphaeae* and then determine if there was a relationship between the two. Because *R. nymphaeae* is a polyphagous and rapidly cycling species and Lemnaceae are common hosts as well as being a rapidly cycling species, it is a model system to study preference and performance characteristics. My results support the conclusion that host plant preference is positively correlated with offspring performance based on the data collected in this study. *Landoltia punctata* was found to be as good as *Spirodela polyrhiza*, but aphids still preferred *S. polyrhiza* over *L. punctata*. However, I found no evidence to suggest that aphids prefer duckweeds with high concentrations of nitrogen. Aphid performance levels were found to be much higher on the duckweeds that contained the lowest concentrations of nitrogen. Therefore, performance could be affected by other compounds and chemicals not looked at in this study.

Aphid mobility

Overall, *R. nymphaeae* are actively mobile. In fact many species of aphids, such as the *R. nymphaeae* which are heteroecious, move between a woody primary host and an herbaceous secondary host (Dixon 1973). Over-crowding, competition and feeding resources may all increase aphid mobility (Scotland 1940; Dixon 1973). In this study, aphids did develop alate individuals when the colonies became overcrowded. In fact, during this experiment, I noticed that if aphid numbers became too immense, they died off. *Rhopalosiphum nymphaeae* are phloem-feeding insects, and must therefore feed by

inserting their stylet into the host plants to suck out the sap. Aphids probe the plants before eating, so some of the movement could be aphids searching for an acceptable host (Scotland 1940).

Aphid host preference

Rhopalosiphum nymphaeae, a polyphagous species, clearly demonstrated a preference among the three species of duckweeds. *Rhopalosiphum nymphaeae* preferred *S. polyrhiza* over *L. punctata* over *L. minor*. Barron (2001) implies that it is likely that genetics, selection and conditioning are all involved in the formation of a preference for a new host, and as such implies that an invasive species could become a preferred species. Data from this study show that the invasive species, *Landoltia punctata* was the second most preferred species.

To address my second research question, one integral factor that did influence preference was familiarity. Other studies have shown that both maternal effects and early larval experience with a host can influence preference (Hopkins 1917; Corbet 1985; Rietdorf and Steidle 2002). Initially aphids preferred the species upon which it was reared, despite it not being a preferred species. However, as experience with other duckweed species grew, aphids switched over to the preferred species, which in this study was *S. polyrhiza*, regardless of the choices offered. Solarz and Newman (2001) when testing milfoil weevils on native and exotic watermilfoils found that early larval experience did not have any effect on host plant preferences. Other similar studies have found that aphids on a preferred host will settle rapidly into feeding and then show little tendency to move (Caillaud and Via 2000), thus having aphids accumulate on the favored host.

Factors other than familiarity may influence an insect's preference. Leen (1998) tested the oviposition preference of the caterpillar, *Uresiphita reversalis*, and found that host plant chemistry and its interaction with natural enemies can influence host preferences. However, because *Uresiphita reversalis* is a holometabolous insect, the host plant chemistry and interaction with natural enemies could be largely influenced by the mother, and not the offspring. Another study showed that host preferences can be modified by maternal effects and conditioning (Barron 2001), another study claims that preference is an adaptation to plant defenses (Papaj and Prokopy 1989; Bernays and Weis 1996). Powell et al. (2006) suggests that aphids may be in contact with nutritionally suitable host plants, yet in the absence of suitable cues the insects will not settle, feed or reproduce.

To test whether a preference is influenced by any of the before mentioned reasons, a study must incorporate measures of both preference and performance. Agrawal et al. (2002) suggests that the use of a host preference and subsequent offspring performance is a form of phenotypic plasticity that may be particularly beneficial for generalist herbivores that use diverse host plants. Thus variable host plants may favor initiation of preference and performance.

Aphid performance

To address my third research question, is aphid host preference positively correlated with performance, I found evidence from this study to support the conclusion that there is a positive relationship. *Rhopalosiphum nymphaeae* preferred and performed well on both *Spirodela polyrhiza* and *Landoltia punctata* in all measures of fitness. This result is quite surprising, given that *L. punctata* is an invasive species. According to the

escape-from-enemies hypothesis, *L. punctata* should lack aphids and provide poor survival and fecundity rates as compared to the native duckweed species, but this was not found to be the case. *Landoltia punctata* has been in the United States for over 70 years giving aphids multitudes of generations to gain experience with it, yet it was only the second most preferred species. This type of disconnect has been seen in other studies comparing native and invasives. *Silene latifolia* which has been in North America for over two hundred years still suffers more enemy damage from herbivores in its native range of Europe than in its introduced range of North America (Wolfe 2002). Bowers et al. (1992) found that when the Baltimore checkerspot (*Euphydryas phaeton*) was given a choice between the native host plant, turtlehead (*Chelone glabra*) or the introduced weed plantain (*Plantago lanceolata*), the butterfly chose the native species. In fact, this study showed that *E. phaeton* preferred and performed best on the native species. Bowers et al. (1992) hypothesized that this preference for the native host species was due to the fact that it contained higher concentrations of iridoid glycosides, which cause the butterfly to be unpalatable to potential predators.

Yet, there are some species that have been able to overcome this problem, and actually prefer and perform better on the invasive species. Milfoil weevils (*Euhrychiopsis lecontei* Dietz) preferred the invasive watermilfoil over the native one, and had faster development rates and reached larger adult sizes than those on the native watermilfoil. This positive relationship is hypothesized to be due to the fact that the invasive watermilfoil has a larger stem diameter and increased oxygen transport compared to the native watermilfoil (Solarz and Newman 2001).

However, a disconnect between aphids preferring and performing well on *L. punctata* suggests that there is still something that does not make it as desirable as the native hosts. It could possibly be that aphids are just not physiologically capable of development on duckweed species not found within their host range (Solarz and Newman 2001). Or perhaps the disconnect is due to genetic differences between the duckweed species. Until as late as 1999, *L. punctata* was placed in the same genus as *S. polyrhiza* (Les and Crawford 1999). Therefore, these two species are extremely similar, yet there may be a specific gene that allows *S. polyrhiza* to be preferred over *L. punctata*.

The results from my study are quite different from many others, including a study examining host-plant preference and performance between the stem-galling fly, *Eurosta solidaginis* and its host goldenrod, *Solidago altissima*. Cronin et al. (2001) found that there was only a weak correlation between preference and performance. They suggested their results were due in part to temporal fluctuations, which are likely to favor a more generalized diet. The data from my study indicate a very specialized diet among the polyphagous *R. nymphaeae*. The differences could also be explained by the fact that the stem-galling fly study was a field experiment, whereas this study was performed completely in a laboratory, which did not undergo temporal fluctuations, nor any of the other abiotic and biotic factors that are present in the field. In a review, Jaenike (1990) discussed that generalist predators are a major cause of mortality in aphids and can have a major effect on host plant use. Since this was a controlled experiment, there were no predators present during the study. Thus, this study could be extremely different if performed in the field. Aphids clearly demonstrated a positive relationship between preference and performance and it is likely due to the fact that the offspring gain

experience of the host plant via maternal effects as well as being born live onto the same host plant.

Another positive correlation of preference and performance was shown in the reproductive output. Aphids reared on the most preferred species, *Spirodela polyrhiza*, produced the most offspring when tested on *S. polyrhiza* (average = 14.4). Aphids reared on the second most preferred host plant species, *L. punctata* also produced a large number of offspring when tested on *L. punctata* (average = 12.9). *Lemna minor* reared aphids were not reproductively successful regardless of which species they were tested on. Jallow and Zalucki (2003) found that on the plant species for which survival was low, smaller pupae were produced, and fecundity was greatly retarded. Jaenike (1990) suggests that environmental factors can contribute to variation in fecundity. These factors include weather, abundance of the host plant, and the presence of conspecific eggs or pheromones (Jaenike 1990). Again, because this study was performed in a laboratory, certain factors such as weather and host abundance were controlled. Therefore, this implies that the presences of conspecific eggs or pheromones could play a large part in fecundity of the aphid.

Aphid growth was also positively linked with host preference. *Rhopalosiphum nymphaeae* progressed through all five instars 88% of the time when tested on the preferred species. Caillaud and Via (2000) showed that an unwillingness to feed on the alternate (less or least preferred) host leads directly to reduced individual fitness, which could help explain the local adaptation among populations in the wild. Overall, the time of development in this study was linked to the duckweed species upon which it was tested, again showing a correlation between preference and performance. Similar studies

have found variations in the amount of time insects required to develop (van Tol et al. 2004), thus showing host plant species can greatly impact the rate of development.

Nitrogen content

To address my fourth and fifth research questions, I found nitrogen content of the duckweeds. The data from this study indicate that host preference is not reflective of host quality. Limited evidence indicates that generalists and specialists may differ in the ability to discriminate among hosts of different quality or in the speed with which decisions are made (Bernays 1998; Bernays and Funk 1999). Although only slight differences were seen in the concentrations of nitrogen among the three duckweed species, *Rhopalosiphum nymphaeae* preferred the nutrient lacking species, *L. minor*. Host-plant nitrogen content is viewed as the ultimate limiting nutrient for most insects, which can result in higher growth and consumption rates in generalist phytophagous insects (Mattson 1980; Prudic et al. 2005). Interestingly though, *Lemna minor*, which was the least preferred species, contained the highest concentration of nitrogen ($P = 0.03$) among the three duckweeds. In herbivorous insects, higher nitrogen levels generally increase larval development and growth rates (Slansky and Feeny 1977; Tabashnik 1982; Prudic et al. 2005).

Consequently, to answer my fifth research question, aphid offspring performance was not directly influenced by nitrogen concentration. Some insects may be compensating for the lack of nitrogen by increasing their food consumption or by concentrating their feeding on the most nitrogen rich plant parts (Slansky and Feeny 1977; Mattson 1980). Duckweeds grown on nutrient-rich water has been shown to have high concentrations of trace minerals, potassium and phosphorus pigments, carotene, and

xanthophylls. The combination of these compounds makes duckweed an especially valuable meal, and in fact duckweed protein has a better array of amino acids than most vegetable proteins (Leng et al. 1995). The combination of these compounds may have allowed aphids tested on *S. polyrhiza* and *L. punctata* to compensate for lower levels of nitrogen.

Conclusions

Many studies have been done to test for host preference and offspring performance in insect species. Some studies that have attempted to show the correlation have either confounded within-and among-population variation or confounded components of preference and performance (Singer et al. 1988). Both behavioral (preference) and physiological (performance) traits are important components of the ability of phytophagous insects to use particular plant species as hosts (Futuyma and Peterson 1985). This study attempted to demonstrate the relationship between preference and performance in terms of familiarity, nutrient content and measures of fitness. The data from this thesis have shown there is a positive relationship between host preference and offspring performance among the aphids that were reared and tested in the laboratory. The two most preferred species, *Spirodela polyrhiza* and *Landoltia punctata*, did yield the highest fitness levels for aphids. However, aphid preference was still stronger for the native species over the invasive species. This shows that there must be more research for measures of preference and performance among native and invasive species to help understand the proximate mechanisms behind preference.

In conclusion, this study showed 1) *R. nymphaeae* do exhibit a host-plant preference, 2) initial host preferences are influenced by maternal effects, 3) there is a

positive correlation between host-plant preference and offspring performance among *R. nymphaeae*, in fact aphids performed better on the two most preferred species, 4) host preferences were not reflective of host quality and 5) offspring performance was not influenced by the nitrogen content of the duckweeds. The study provides evidence that host preference and offspring performance can be positively correlated. Further studies should examine the mechanisms behind the rearing effect, i.e. familiarity effect and also the proximate mechanisms in determining aphid preference. Also any study to increase the awareness and understanding of host-plant preference in aphids would be beneficial in helping to prevent further economic damage caused by these pests on important agricultural crops throughout the world.

Table 1. Host preference of aphids among the three duckweed species. The data table represents the aphids' overall host preference. The preferred species is shown in bold font. N represents the number of aphids that chose each of those species in that test. The column labeled χ^2 shows the chi-square values. (* indicates $P < 0.05$, ** indicates $P < 0.001$)

Choices	N	χ^2
<i>Spirodela polyrhiza</i>	39	14.225**
<i>Landoltia punctata</i>	8	
<i>Landoltia punctata</i>	35	6.125*
<i>Lemna minor</i>	14	
<i>Spirodela polyrhiza</i>	37	10.939**
<i>Lemna minor</i>	8	

Table 2. Maternal effects on aphid preferences. The first column shows the pairs of choices that were presented to the aphids. Preferred species are shown in bold. The first ratio under column N represents the number of aphids that were on the reared species compared to the number not on that species. The second ratio represents the expected values against which the observed ratios were tested. Aphids initially preferred the species of duckweed they were most familiar with, hence the species they were reared on.

Choices	N	χ^2
<i>Spirodela polyrhiza</i> <i>Landoltia punctata</i>	15:1 vs. 39:8 8:8 vs. 8:39	10.143*
<i>Spirodela polyrhiza</i> <i>Lemna minor</i>	13:3 vs. 37:8 13:4 vs. 8:37	11.626*
<i>Landoltia punctata</i> <i>Lemna minor</i>	13:4 vs. 35:14 13:2 vs. 14:35	14.929**

* indicates $P < 0.05$, ** indicates $P < 0.001$

Table 3. Generation times (T_c) of *Rhopalosiphum nymphaeae*. Generation times of the aphids were significantly affected by the duckweed species on which they were tested ($F_{2,78} = 1.576$, $P = 0.0378$).

Rearing Species	Testing Species	T_c (days)(mean \pm SE)
<i>Landoltia punctata</i> *	<i>Landoltia punctata</i>	10.13 \pm 0.93
	<i>Lemna minor</i>	5.75 \pm 0.75
	<i>Spirodela polyrhiza</i>	8.77 \pm 1.99
<i>Lemna minor</i>	<i>Landoltia punctata</i>	10.04 \pm 0.71
	<i>Lemna minor</i>	6.5 \pm 0.5
	<i>Spirodela polyrhiza</i>	12.11 \pm 1.64
<i>Spirodela polyrhiza</i>	<i>Landoltia punctata</i>	5.69 \pm 1.68
	<i>Lemna minor</i>	6.62 \pm 0.55
	<i>Spirodela polyrhiza</i>	10.52 \pm 1.80

Table 4. Intrinsic rates of increase (r_{\max}) for *Rhopalosiphum nymphaeae*. There was a significant difference among aphid intrinsic rates of increase based upon the species of duckweed on which they were tested ($F_{2,78} = 6.775$, $P < 0.0001$).

Rearing Species	Testing Species	r_{\max} (mean \pm SE)
<i>Landoltia punctata</i> *	<i>Landoltia punctata</i>	0.249 \pm 0.01
	<i>Lemna minor</i>	0.053 \pm 0.05
	<i>Spirodela polyrhiza</i>	0.218 \pm 0.02
<i>Lemna minor</i>	<i>Landoltia punctata</i>	0.140 \pm 0.03
	<i>Lemna minor</i>	0
	<i>Spirodela polyrhiza</i>	0.164 \pm 0.02
<i>Spirodela polyrhiza</i>	<i>Landoltia punctata</i>	0.171 \pm 0.03
	<i>Lemna minor</i>	0.052 \pm 0.03
	<i>Spirodela polyrhiza</i>	0.197 \pm 0.03

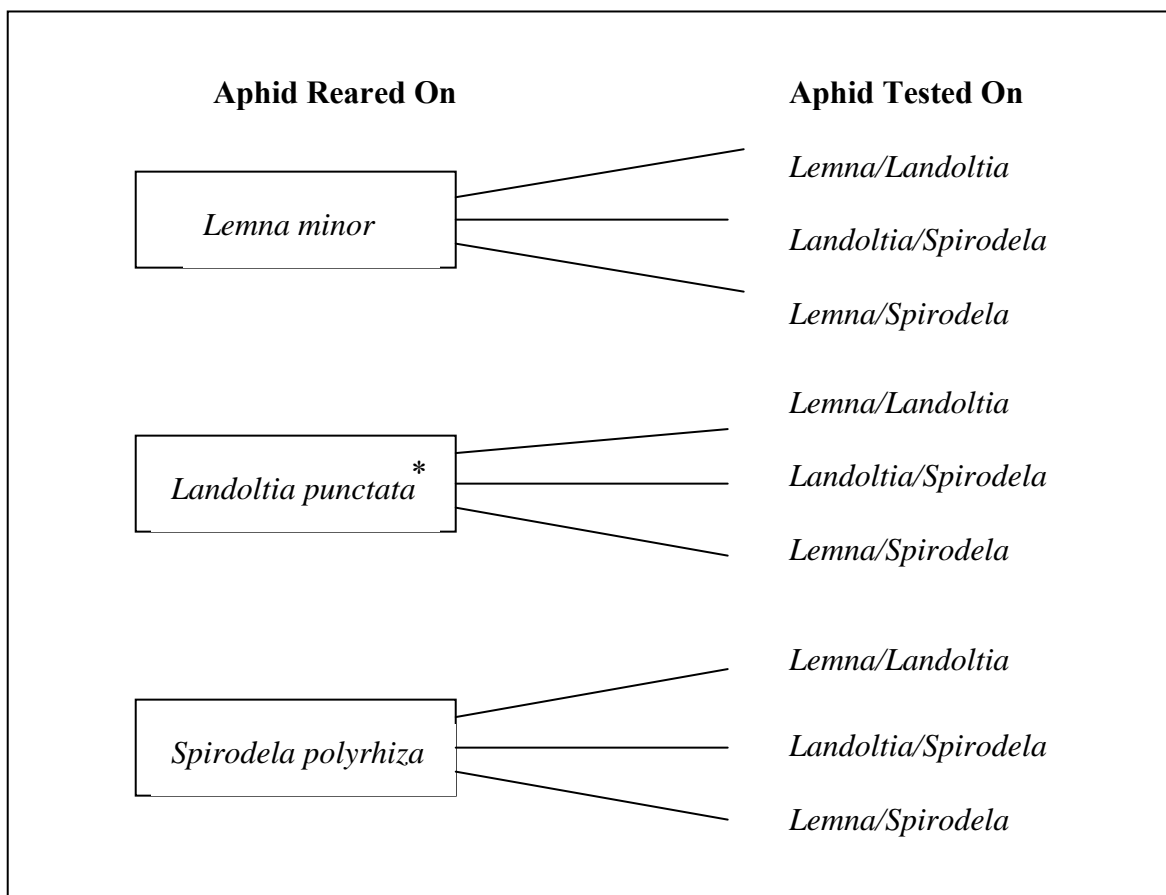


Figure 2. Experimental design for preference study. The combinations of duckweed species that were placed in the film canisters are shown on the right in the diagram, for a total of nine treatment levels. *Landoltia punctata* is the invasive plant species and is denoted with the *.

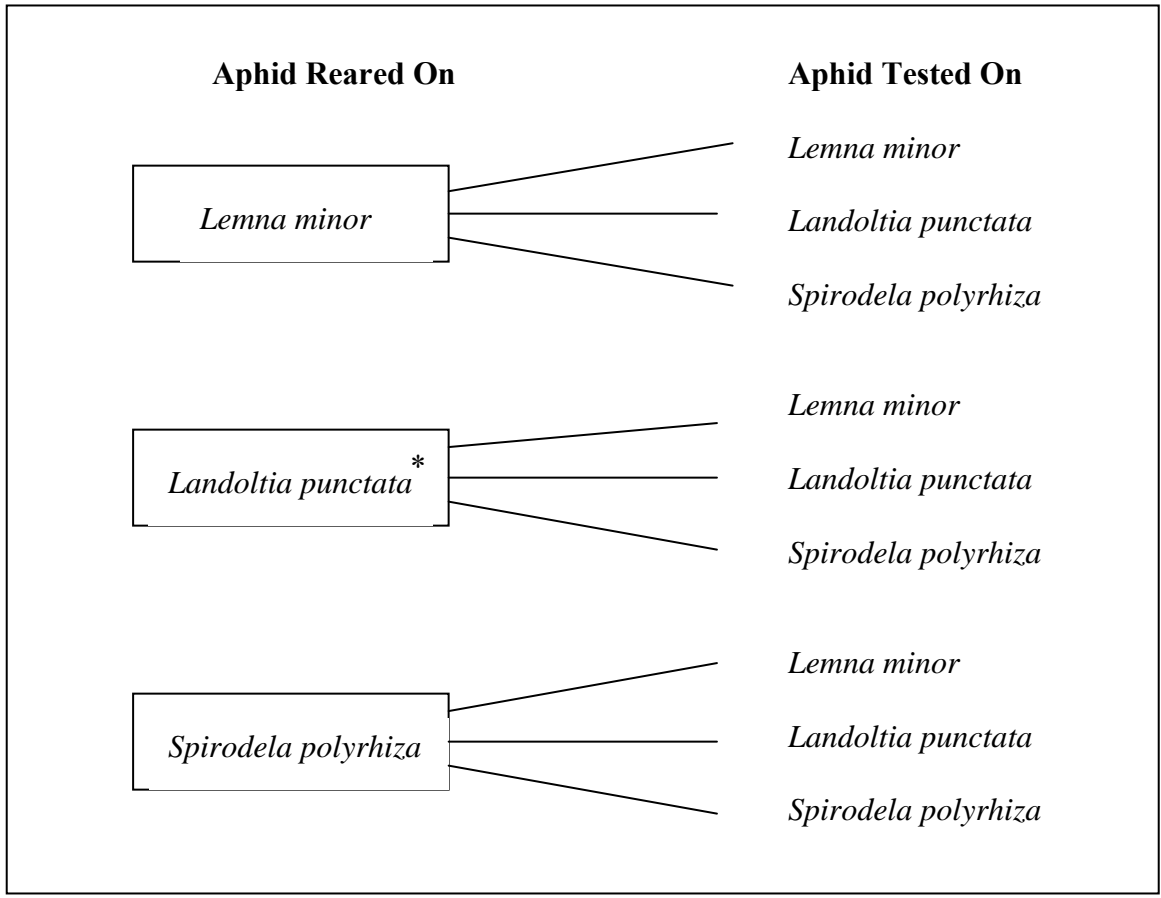


Figure 3. Experimental design for performance study. The boxes on the left represent the tubs in which the aphids were reared. The column on the right represents the nine treatments upon which each first instar aphid was placed. * denotes the invasive species.

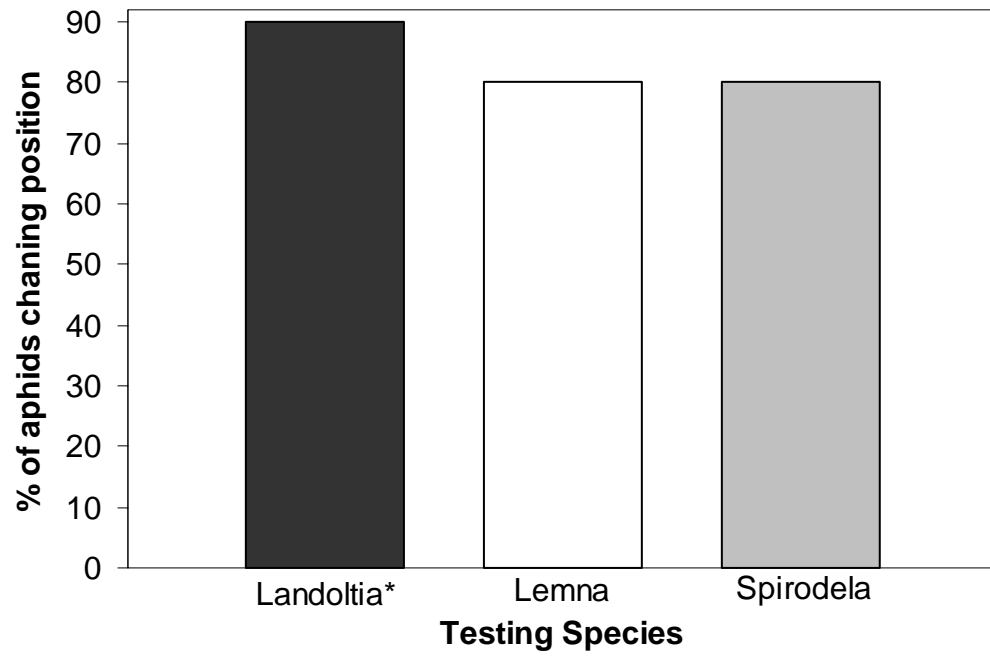


Figure 4. Mobility of *Rhopalosiphum nymphaeae*. The bars represent the overall percentage of aphids that changed position between readings.

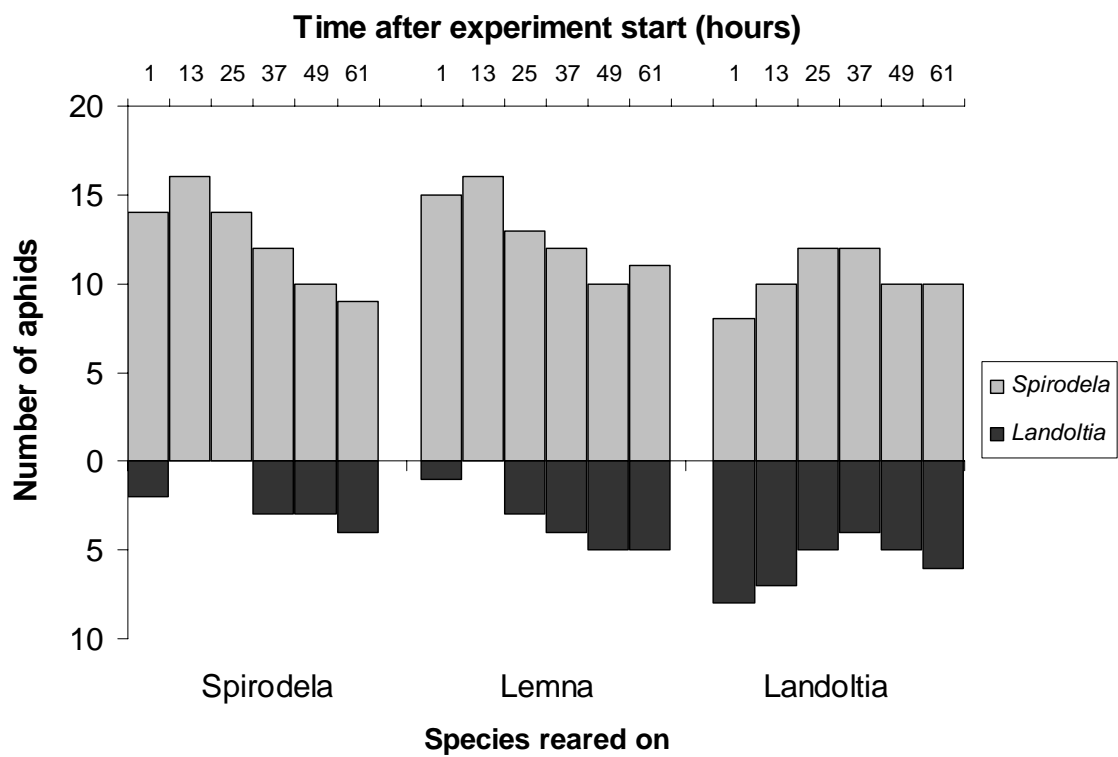


Figure 5A. Aphid preference between *Spirodela polyrhiza* and *Landoltia punctata*.

Graphs 5A-5C show the number of aphids on the duckweed species at each reading of the study.

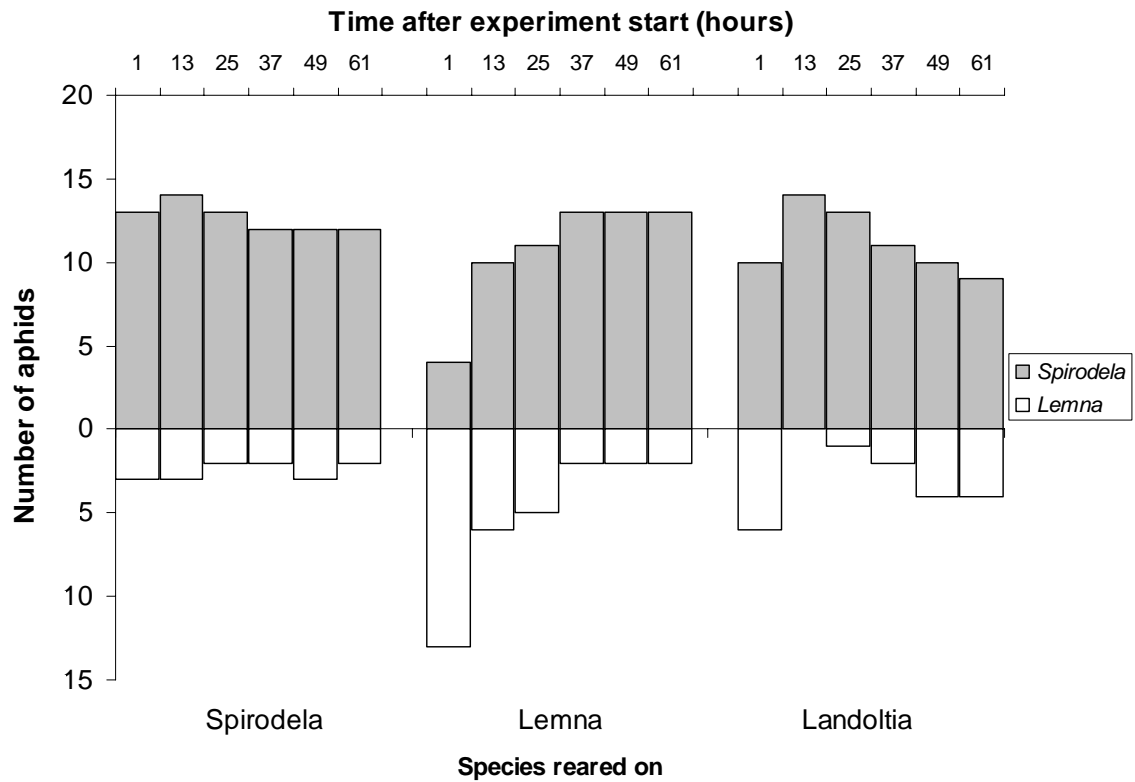


Figure 5B. Aphid preference between *Spirodela polyrhiza* and *Lemna minor*.

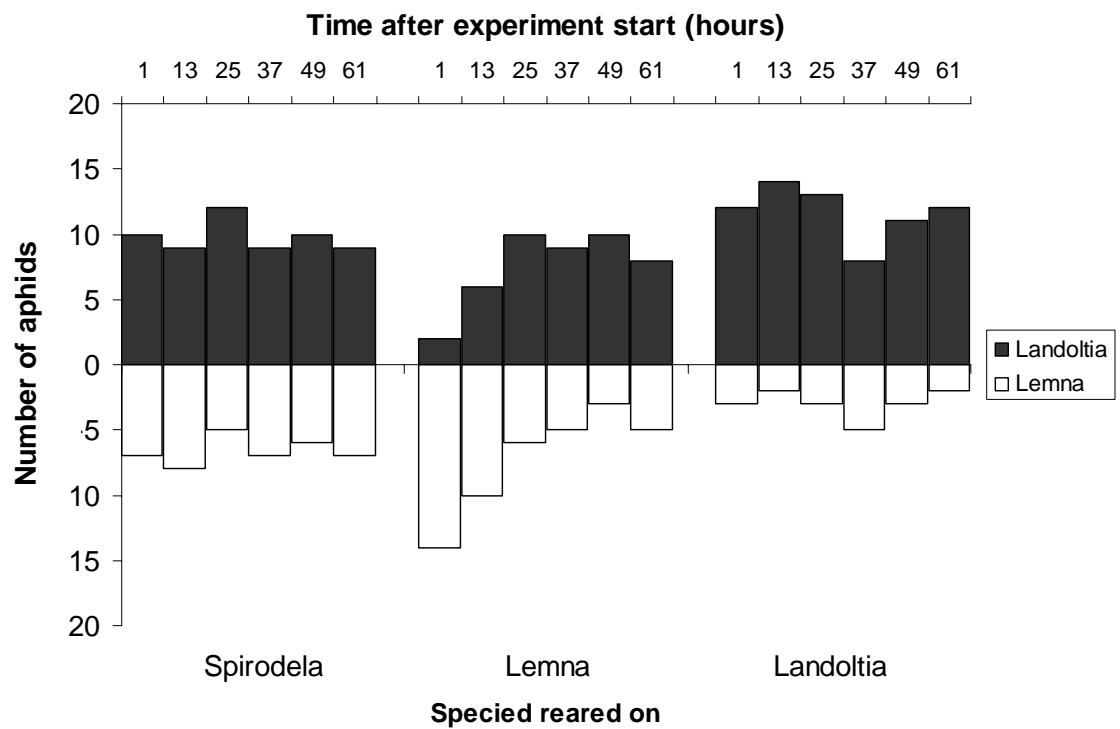


Figure 5C. Aphid preference between *Landoltia punctata* and *Lemna minor*.

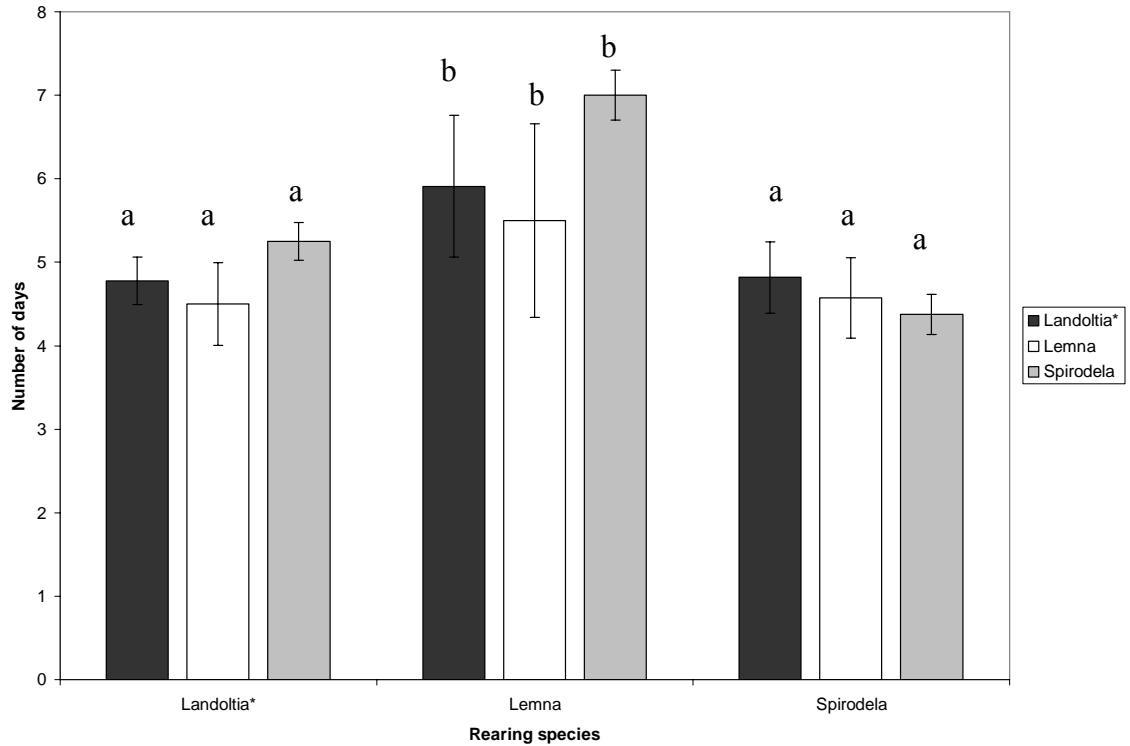


Figure 6. Number (mean \pm SE) of pre-reproductive days for *Rhopalosiphum nymphaeae*. The number of pre-reproductive days was significantly different for aphid reared on different species of duckweed ($F_{2,78} = 2.83$, $P = 0.0087$). * denotes *Landoltia punctata* as the invasive species.

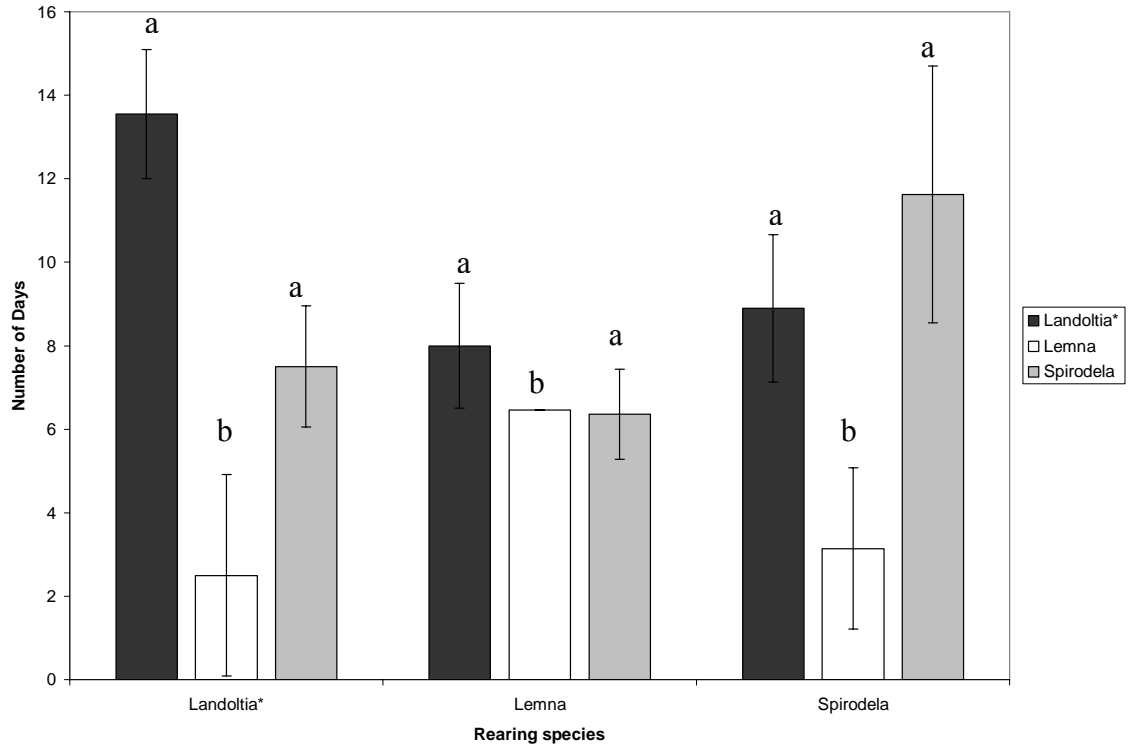


Figure 7. Number (mean \pm SE) of reproductive days for *Rhopalosiphum nymphaeae*. The species of duckweed on which the aphid was tested had a significant effect on the number of reproductive days ($F_{2,78} = 2.79$, $P = 0.0081$).

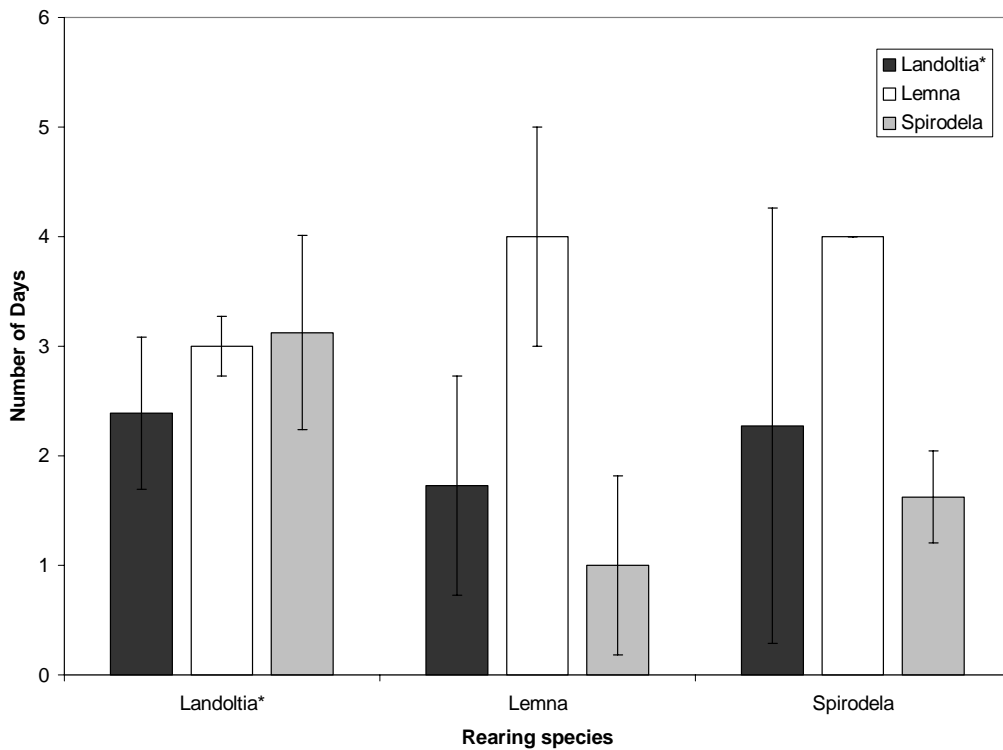


Figure 8. Number (mean \pm SE) of post-reproductive days for *Rhopalosiphum nymphaeae*.

There were no significant effects for aphids in the number of post-reproductive days

($F_{2,78} = 1.04$, $P = 0.42$).

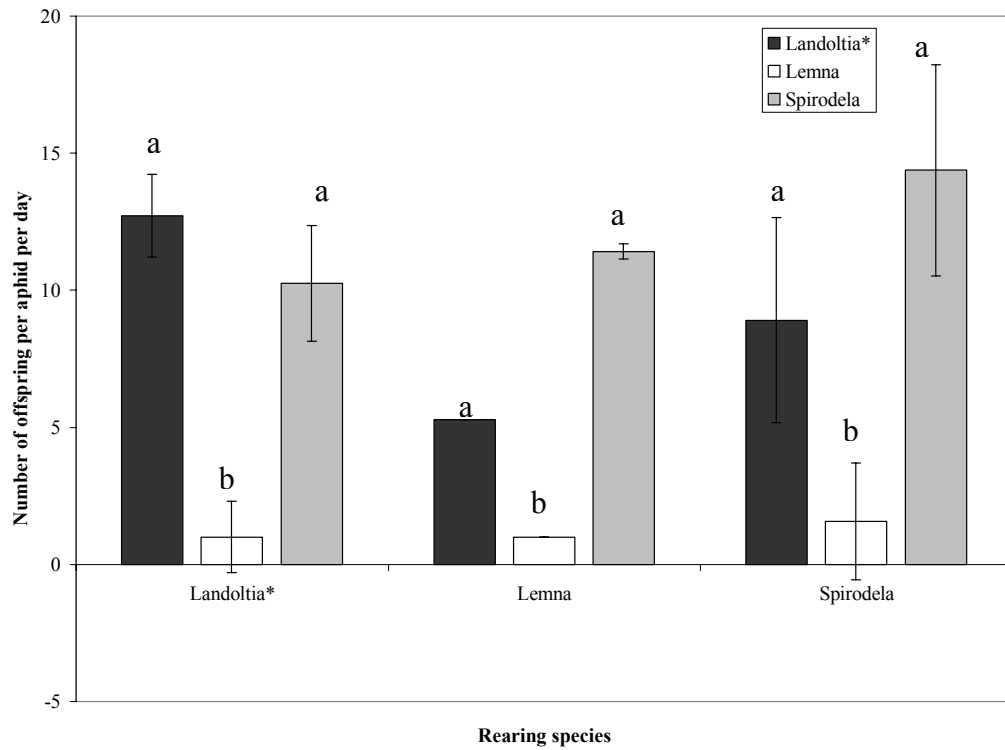


Figure 9. Offspring production of *Rhopalosiphum nymphaeae*. Bars represent the mean \pm SE. There was a significant difference in the duckweed test species for the number of offspring produced by each aphid ($F_{2,78} = 3.30$, $P = 0.02$).

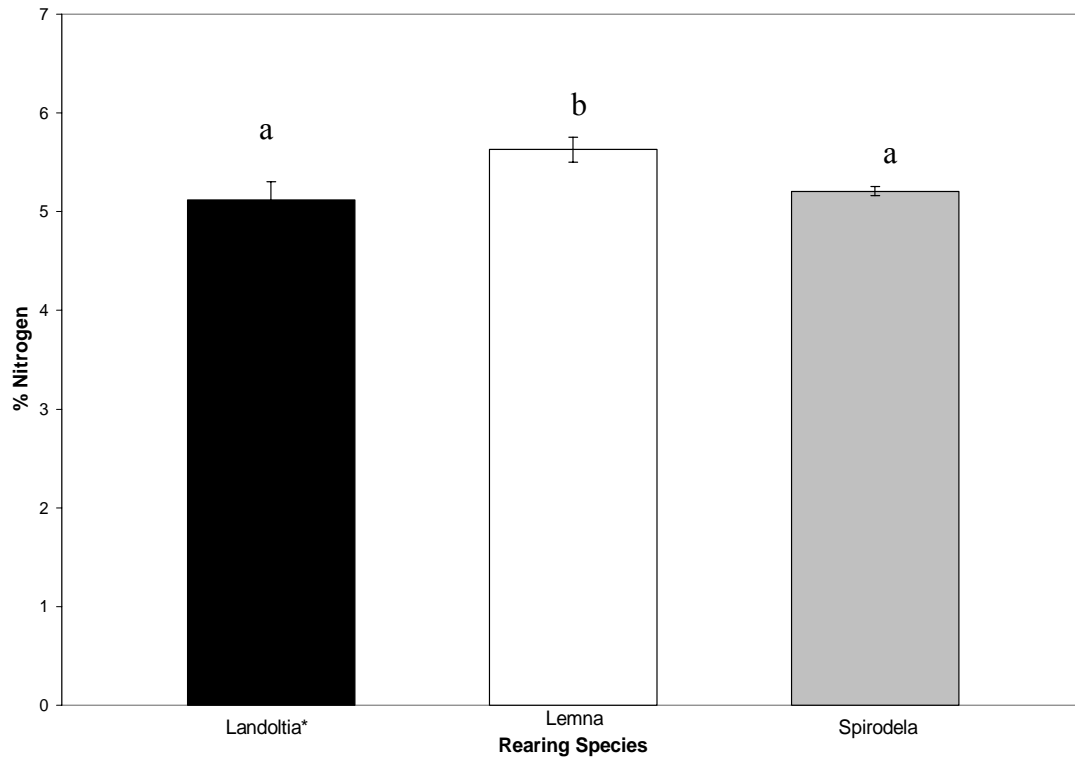


Figure 10. Dry mass percentage (mean \pm SE) of nitrogen in duckweed tissue. The percentage of nitrogen was significantly highest in *Lemna minor* ($F_{2,18} = 4.14$, $P = 0.03$). Bars with different letters are significantly different ($P < 0.05$).

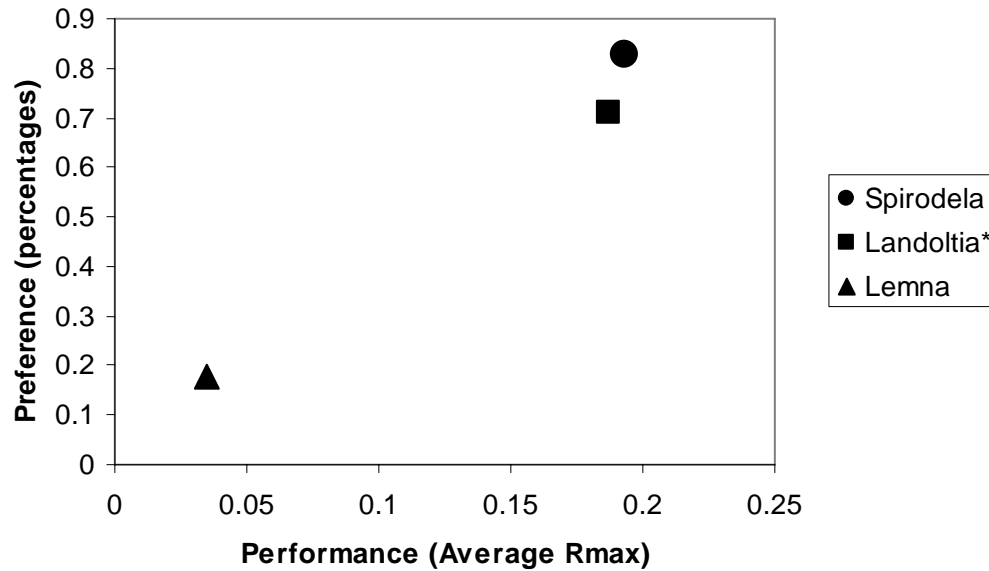


Figure 11. Relationship of aphid preference and offspring performance. Performance values are the average r_{\max} values calculated (Table 4) and the preference values are the percentages from the preference tests (Table 1). Aphids on *Spirodela polyrhiza* and *Landoltia punctata* had higher fitness levels than aphids on *Lemna minor*. Because *S. polyrhiza* was the most preferred host plant, it is slightly higher than *L. punctata* on the graph.

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APPENDIX A
THREE-WAY ANALYSES OF DURATION
OF FIRST INSTAR (BIRTH TO 1ST MOLT)

The three factors are reared on, *A* (*Spirodela polyrhiza*, SP; *Landoltia punctata*, LP; *Lemna minor*, LM); tested on, *B* (*Spirodela polyrhiza*, SP; *Landoltia punctata*, LP; *Lemna minor*, LM); and percentage of aphids that molted in one day or less, *C* (variables are the same for tables 5 – 8). The duckweed species reared on had the largest effect ($G_6 = 52.5$, $P = 0.00$) for this aphid instar duration.

Reared on (a = 3) A	Tested on (b = 3) B	Percentage molted (c = 2) C
SP	SP	78%
	LP	93%
	LM	89%
LP	SP	43%
	LP	75%
	LM	86%
LM	SP	20%
	LP	16%
	LM	42%

APPENDIX B

THREE-WAY ANALYSES OF DURATION OF

SECOND INSTAR (1st MOLT TO 2nd MOLT)

Aphids that were reared on and tested on the same species had the quickest molt times for the duration of the 2nd instar. There was a significant difference in the duration of the instar for the duckweed species on which the aphid was reared ($G_6 = 15.2$, $P = 0.02$).

Reared on (a = 3) A	Tested on (b = 3) B	Percentage molted (c = 2) C
	SP	83%
SP	LP	58%
	LM	78%
	SP	86%
LP	LP	85%
	LM	62%
	SP	44%
LM	LP	56%
	LM	86%

APPENDIX C

THREE-WAY ANALYSES OF DURATION OF

THIRD INSTAR (2nd MOLT TO 3rd MOLT)

Neither the rearing species nor the testing species had an affect on the duration of the instar ($G_6 = 10.9$, $P = 0.09$).

Reared on (a = 3) A	Tested on (b = 3) B	Percentage molted (c = 2) C
SP	SP	95%
	LP	96%
	LM	96%
LP	SP	79%
	LP	86%
	LM	93%
LM	SP	85%
	LP	85%
	LM	45%

APPENDIX D

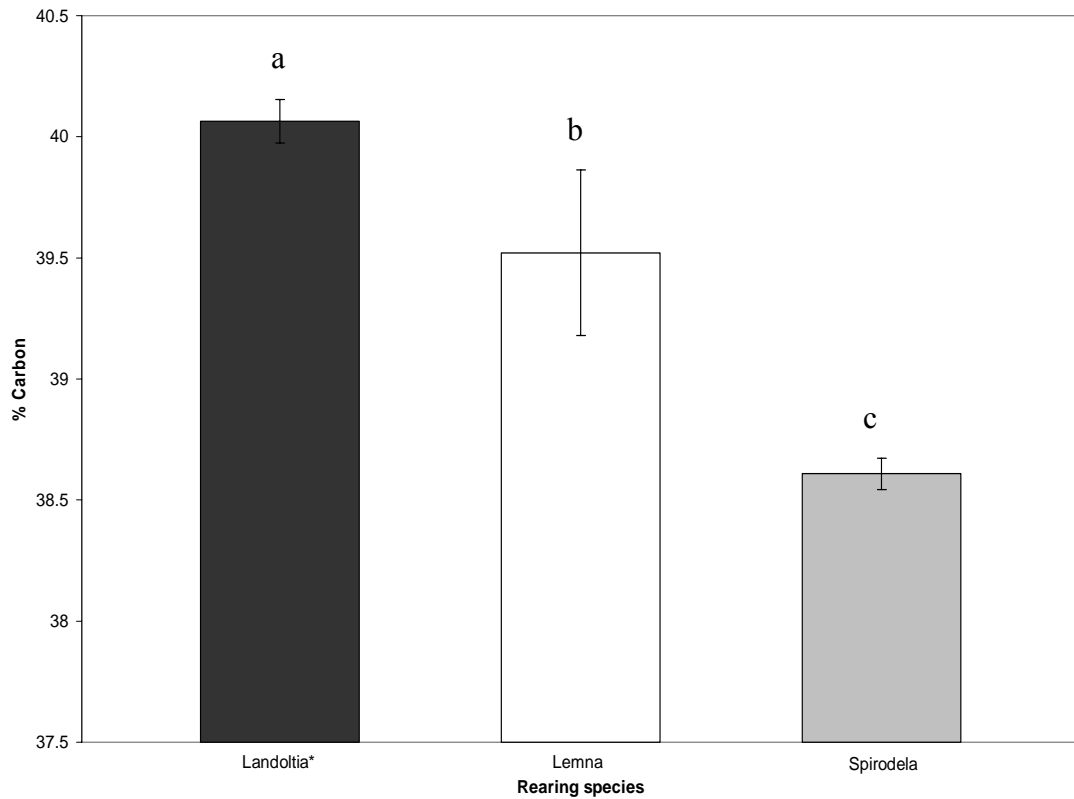
THREE-WAY ANALYSES OF DURATION OF

FOURTH INSTAR (3rd MOLT TO 4th MOLT)

The duration of the 4th instar was affected by the species of duckweed on which the aphid was tested ($G_{10} = 448.6$, $P = 0.00$).

Reared on (a = 3) A	Tested on (b = 3) B	Percentage molted (c = 2) C
SP	SP	94%
	LP	96%
	LM	95%
LP	SP	85%
	LP	69%
	LM	94%
LM	SP	61%
	LP	78%
	LM	63%

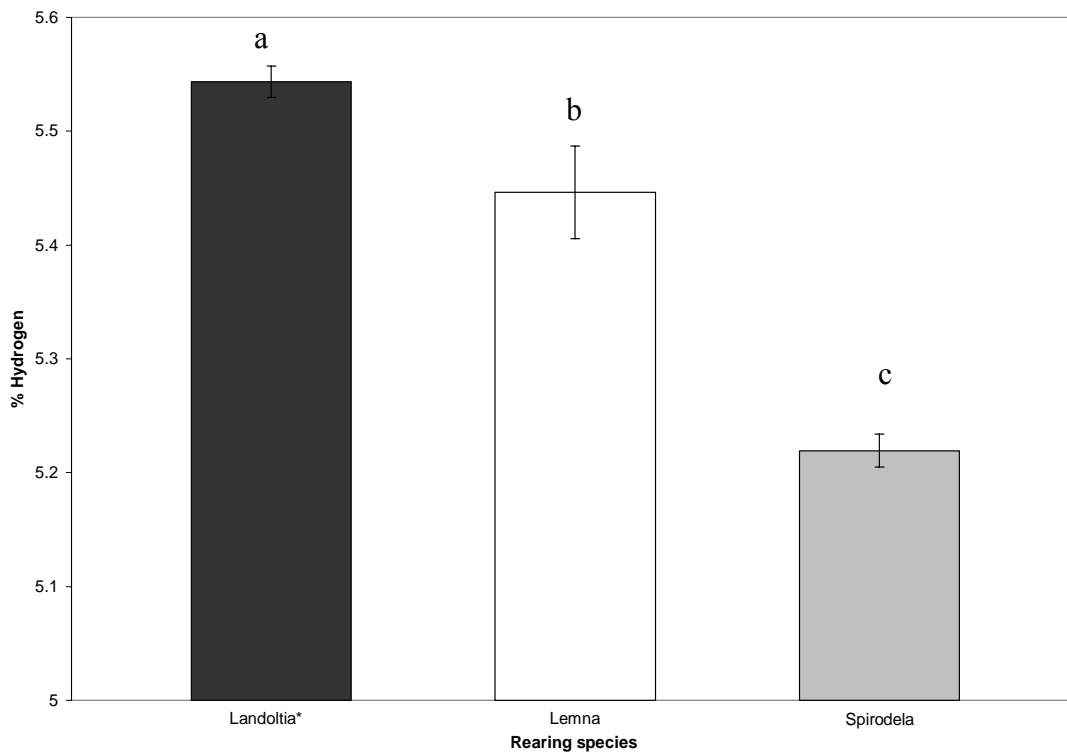
APPENDIX E
DRY MASS PERCENTAGE (MEAN \pm SE) OF
CARBON IN DUCKWEED TISSUE



Carbon was significantly the highest in *Landoltia punctata* ($F_{2,18} = 18.9$, $P = 0.00$).

Different letters above the bars show significant differences ($P < 0.05$).

APPENDIX F
DRY MASS PERCENTAGE (MEAN \pm SE) OF
HYDROGEN IN DUCKWEED TISSUE



Landoltia punctata contained the highest percentage of hydrogen ($F_{2,18} = 40.8$, $P = 0.00$).

Different letters above the bars show significant differences ($P < 0.05$).