

**Observations on the Ecology and Behavior of
Macroseius biscutatus, an Obligate Pitcher Plant Mite**

by
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Keywords: *Macroseius biscutatus*, *Pinus palustris* pitcher plant wetland, *Sarracenia*, smoke

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Abstract

The current conservation status of arthropods symbiotically associated with tall-form *Sarracenia* spp. is poorly known. Yet these unique organisms and their associations are disappearing due to habitat loss, degradation and fragmentation. A survey was conducted of several genera of pitcher inhabitants from several sites in Alabama and Florida. The results of this survey indicate the possible extirpation of *Exyra semicrocea* from small, heavily managed sites, and that the presence of *Exyra* larvae is negatively associated with mite presence although dipteran associates are positively correlated with mites. Furthermore, I present experimental evidence that provides a possible explanation for the continued survival of *Macroseius biscutatus*, a pitcher plant dwelling acarid mite, in habitats where other pitcher inhabitants may have been extirpated. My experiments indicate that *Macroseius biscutatus* responds to cues indicating fire (i.e. smoke). Increased mite movement, including pitcher exit, at the time of fire may allow mites to locate refugia. In conclusion, while intense fire management in small wetland remnants seems to negatively impact *E. semicrocea*, these fires do not seem to affect *M. biscutatus*.

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Model was fit with restricted maximum likelihood function. AIC = 424.1958, BIC = 436.4091, logLik = -207.0979.60

Introduction: Conservation of Pitcher Plant Arthropod Associates

Macroseius biscutatus Muma, Denmark and Chant and other pitcher plant arthropods present a case for arthropod conservation that finds a foundation in utilitarian and aesthetic arguments but is only fully realized within a Leopoldian scope. Arthropods in general are indispensable. Arthropods fulfill vital and irreplaceable roles in our ecosystems and to the economies that depend on those. Beyond ecological and utilitarian value, arthropods are unique and often aesthetic outcomes of past evolution and the basis for future evolution. When considered as a phylum, arthropods are ubiquitous, yet many species are experiencing extinction pressure, especially in threatened habitats. Arthropod conservation is an underappreciated concern in a tradition of focusing on large charismatic animals. Conservation efforts need to be extended to declining arthropods that are endemic to habitats experiencing anthropogenic pressure. One such type of habitat is pitcher plant wetlands, which occur within *Pinus palustris* Mill. (Longleaf Pine) forests of the southeastern United States. Many endemic arthropods are faced with a threat of extinction due to increasing alteration and fragmentation of their habitat. These species are irreplaceable, and it is imperative that we protect them.

Debates about human relationships with ecosystems and with particular species have centered on aesthetic and pragmatic arguments. At the turn of the twentieth century, Muir (1901) and Pinchot (1910) championed a similar presupposition, nature should be preserved, but with diametrically opposed applications. Muir (1901) maintained the need for wilderness based on an ethical sense of altruism motivated by the overwhelming aesthetics of the sublime. Muir (1901)

demonstrates in his writing that wilderness exists principally apart from man, but its preservation preserves man's well being. Pinchot (1910) on the other hand, did not separate wilderness from human culture. He sought to save wild lands for wise use of natural resources found on those lands. His argument was that judicious use of wild lands increases prosperity and therefore wellbeing. Muir's ethic has come to be known as preservation, and Pinchot's ethic has come to be known as conservation. Leopold synthesized these views in the land ethic. The land ethic asserts that nature has a right to exist which includes the premise that humans have an ethical obligation to nature. Leopold did not create boundaries between man and nature as did Muir (Leopold 1949). A Leopoldian synthesis is more broadly applicable than Muir's or Pinchot's, since it requires neither the sublime nor the utilitarian.

Many arguments for arthropod conservation arise from utilitarian ethics. Arthropods are a dominant taxon, meaning individuals occur at a high number and have a high ecological impact (Gaston and Fuller 2007). Economic impacts of arthropods apply in areas such as pasture land maintenance and pest control (Losey and Vaughan 2006). Arthropods increase the quality of pasture land. For example, dung burying beetles increase nutrient cycling in pasture land and subsequently the quality of the beef those pastures yield (Losey and Vaughan 2006). Arthropods contribute to pest control as bio-control agents as in the cases of *Salvinia molesta* Mitchell (Kariba-Weed), a floating plant, in Australia (Room et al. 1981) and *Phenacoccus manihoti* Matile-Ferrero (Cassava Mealybug) in Africa (Norgaard 1988). Arthropod conservation is increasingly coming to the attention of scientists and the public. Loss of arthropod biodiversity and biodiversity in general is a serious conservation concern and a potential monetary loss for human society. The potential monetary value has been referred to as option value (Edwards and Abivardi 1998). A well-known example of option value is illustrated by *Taxus brevifolia* Nutt.

(Pacific Yew). Pacific Yew trees were inadvertently preserved as part of *Strix occidentalis* subsp. *caurina* Merriam (Northern Spotted Owl) habitat, but Pacific Yew trees proved to be a natural source of taxol, a cancer fighting compound (Kelsey and Vance 1992).

Beyond option value, the human race depends on ecosystem services provided by arthropod biodiversity (Worm et al. 2006). Arthropods are the most diverse and abundant group of multi-cellular organisms, with astonishing numbers of both species and individuals (Ødengaard 2000) adapted to a vast array of environments. Arthropods are a critical tier of consumers in ecosystems (Pyle et al. 1981).

Arthropod diversity has been reasonably estimated as 4.8×10^6 total species. While this number is considerably smaller than previous estimates (Ødengaard 2000), it is still large. Dillard (1979, 1999), a Pulitzer Prize winning writer, has established through subjective demonstration that numbers this large cannot be meaningfully imagined. Numbers for arthropod diversity are more easily quoted than conceptualized. Nevertheless, the numbers are declining. Stork (2009) estimates insect extinction to range between 7,000 and 14,000 species of insects since seventeenth century. In more recent history, the IUCN Red Lists include 61 species of insects that are extinct and over half of those species occurred in the United States (Wagner and Driesche 2010). The rate of insect extinction is a concern for conservationists specifically within the United States, especially since it has been under reported (Berenbaum 2008). Insects are not the only arthropods facing extinction pressures. Mites, like insects, are virtually ubiquitous but certain species face extinction pressure due to habitat loss. Tixier and Kreiter (2009) noted that the number of phytoseiid mite species endemic to “hot spots” (ecosystems possessing high species diversity and experiencing large amounts of habitat loss) warranted concern.

Endemic species typically face extinction because of habitat loss and the cascading effects of extinction. Cascading extinction is a specific concern since it can affect species with closely related niches (Dunne and Williams 2009) or tightly bound ecological interactions (Koh et al. 2004). For example, lepidopteran population sizes and distributions are tightly bound to their host plant populations. Obligate lepidopteran populations collapse in synchrony with their host plants (Pyle et al. 1981). The tight ecological relationships between host plants and lepidopterans illustrates the potential for far reaching consequences of extinction.

Extinction of arthropods, specifically insects, has been a documented concern since the 19th century (Pyle et al. 1981), and remains a concern today. However, even within insects there appears to be a bias to track showy types of insects (Bossart and Carlton 2002). Bossart and Carlton (2002) compared number of species known in a taxon to number of species listed by Natural Heritage programs. They note that Natural Heritage programs have listed comparatively more lepidopterans (butterflies and moths) and odonates (dragonflies and mayflies) than coleopterans (beetles), hymenopterans (wasps and bees), and hemipterans (true bugs). However, there is little of evidence showing lepidopterans and odonates are especially threatened (Bossart and Carlton 2002). A bias toward larger species is not confined to insects alone. Within the arachnids, mites are often left out of biological studies due to their small size (Fashing 1998).

Degradation of habitat and the disappearance of species is a point of serious concern for land managers and conservation programs. Longleaf Pine habitats are disappearing in the southeastern United States (Gilliam and Platt 2006, Van Lear et al. 2005). These fire maintained habitats are disappearing at alarming rates. Longleaf Pine forests covered an estimated 3.0×10^7 hectares before extensive human encroachment but now cover less than 1/25th of their previous range (Gilliam and Platt 2006).

Sutter and Kral (1994) noted the inadequate conservation of non-alluvial systems within Longleaf Pine communities, such as pocosins and wet-pine savannahs. These habitats and others like them are rapidly diminishing in size and abundance in the Coastal Plain (Sutter and Kral 1994). This is a disturbing observation since Longleaf Pine communities contain 1/3 of the rare plants found in the southeastern Coastal Plain (Sutter and Kral 1994). Longleaf Pine communities are amazingly diverse, containing up to 40 species of plants per square meter and as many as 140 species per thousand square meters (Outcalt 2000). Many species found within Longleaf Pine communities, including 96 plant species alone, are known to be endemic (Outcalt 2000). In addition to plants, animals, both vertebrate and invertebrate, are known to be endemic to Longleaf Pine communities. Notable vertebrates found in Longleaf Pine forests include *Drymarchon corais couperi* Holbrook (Indigo Snakes) and *Gopherus polyphemus* Daudin (Gopher Tortoises) (Outcalt 2000). Invertebrates include many types of endemic arthropods, few of which have been reported (Rymal and Folkerts 1982).

Pitcher plants and other carnivorous plants occur in wet pine savannahs within the Longleaf Pine forest (Keddy et al. 2006). In the late 1770s, naturalist William Bartram traveled through the southeastern United States. He reported traveling over country that included “expansive savannas” between “Talasse” and Mobile, Alabama (Bartram 1996). Elsewhere in his writings, Bartram’s statements and observations of *Sarracenia* L. (pitcher plants) give further credence to the abundance of the habitat at the time he was writing.

Pitcher plant wetlands are open and sunny habitats in wet sandy soils with low pH, and often overlying an impermeable layer of soil called a hardpan. These wetlands are habitat for many species of carnivorous plants as well as their inhabitants (Rymal and Folkerts 1982, Folkerts 1999). The ecological conditions in these wetlands are commensurate with those

outlined by Juniper et al. (1989); the soil is nutrient poor, water is rarely limited, and there is little competition for sunlight (Juniper et al. 1989).

Sarracenia are passive carnivorous plants with leaves, called pitchers, which trap and subsequently absorb nutrients from prey. Pitchers attract prey via nectar secretion, color and pattern. Nectar is secreted in the peristome area above the opening to the cone shaped trap below (Joel 1988, Juniper et al. 1989). Pitchers are divided into four zones: attractive zone, conductive zone, glandular zone, and detentive zone. Each zone has specific morphological characteristics (Juniper et al. 1989). The attractive zone features nectar secretions and nectar guides. Nectar guides within *Sarracenia* often include nectariferous lines as well as contrasting patterns (Joel 1988). The conductive zone has waxes, downward pointing trichomes and cellular projections. The glandular zone, present in some *Sarracenia*, has enzyme secreting glands. The detentive or digestive zone features long stiff trichomes (Juniper et al. 1989).

Sarracenia, native from Florida to Canada, is a widely studied genus of carnivorous plants (Ellison et al. 2004). This genus contains *S. purpurea* L. (Purple Pitcher Plant), *S. psittacina* Michx. (Parrot Pitcher Plant), *S. flava* L. (Yellow Pitcher Plant), *S. rubra* Walt (Sweet Pitcher Plant), *S. leucophylla* L. (White Topped Pitcher Plant), *S. oreophila* (Kearney) Wherry (Green Pitcher Plant), *S. alata* Alph. Wood (Yellow Trumpets), and *S. minor* Walter (Hooded Pitcher Plant) (Rymal and Folkerts 1982). Recently, Purple Pitcher Plants and Yellow Pitcher Plants have been recognized as newly introduced in Europe (Adlassnig et al. 2010). Purple Pitcher Plants and Parrot Pitcher Plants are notably shorter than the other species which are called tall-form pitchers (Folkerts 1999).

Plant carnivory has been the object of much investigation. Givnish et al. (1984) reported that phytocarnivory was favored in habitats in which nutrients available in the soil were limited while other resources necessary for photosynthesis (i.e. water and light) were not. However, recent work has shifted focus to the adaptive significance of root construction. *Sarracenia* do not invest heavily in roots compared to other plants that compete for similar niches. In essence, *Sarracenia* employ a strategy alternate to producing aerenchyma (air space in root tissue) in wet soils, by producing shallow roots and supplementing nutrient uptake with prey capture (Brewer et al. 2010). Pitchers are costly to plants in terms of nutrient investment. It takes pitcher plant leaves longer to produce a net gain in carbon than leaves of other plants (Karagatzides and Ellison 2009).

Pitchers are valuable resources to pitcher plants and represent resources to organisms uniquely adapted to life in pitchers (Rymal and Folkerts 1982). Arthropod inhabitants regularly found in pitchers are Hymenoptera (wasps), Diptera (flies), Lepidoptera (butterflies and moths), and Acarina (mites) (Adlassing et al. 2010, Jones 1921, Rymal 1980, Rymal and Folkerts 1982). Diptera include *Wyeomyia smithii* Coquillet, *W. haynei* Dodge (Darsie and Williams 1976), *Mectrocnemus knabi* Coquillet, *Fletcherimyia celerata* Aldrich, *Blaesoxipha rileyi* Aldrich, *Bradysia macfarlanei* Jones, and an un-described chloropid species (Rymal and Folkerts 1982). Hymenoptera include *Isodontia mexicana* Saussure, *Isodontia philadelphica* Lepeletier de Saint Fargeau, and *Chlorion harrisi* Fernald (Rymal and Folkerts 1982). Lepidoptera include *Exyra ridingsi* Riley, *E. semicrocea* Guenée and *E. fax* Grote (Jones 1921; Rymal and Folkerts 1982). Acarina include *Sarraceniopus gibsoni* Nesbit, *S. hughesi* Hunter and Hunter, *S. nipponensis* Tagami (Tagami 2004) and *Macroseiopus biscutatus* (Muma and Denmark 1967; Rymal and Folkerts 1982).

Hymenopteran and dipteran inhabitants use pitchers to rear their young. The facultatively associated species *I. mexicana* modifies pitchers and fills them with provisions for its young (Rymal and Folkerts 1982). Among dipteran inhabitants within southeastern tall-form pitchers, sarcophagids in the genus *Blaesoxiphia* are most abundant (Rymal and Folkerts 1982). *Blaesoxiphia* (Sarcophagidae) have evolved adaptations for pitcher plant leaves. In addition to being associated with *Sarracenia*, other sarcophagid species use *Nepenthes* L., a genus of pitcher plants found in Old World tropical regions (Dahlem and Naczi 2006). Sarcophagid flies larviposit into pitchers and larvae feed on prey masses until they mature. When ready to pupate, sarcophagid larvae leave their pitcher and form puparia in the soil (Dahlem and Naczi 2006).

Exyra, obligate inhabitants, have a number of adaptations for life in pitcher plant leaves. Their life history has been well investigated (Rymal and Folkerts 1982; Stephens and Folkerts 2012). *Exyra* lay eggs in *Sarracenia* pitchers and, after hatching, larvae girdle the pitchers and cause wilting. Wilting closes the pitcher orifice and most likely protects *Exyra* from predation but excludes further prey capture by a girdled leaf. Predators include dipterans, parasitic hymenoptera, viral pathogens, bacterial and fungal infection, and some vertebrates (Rymal and Folkerts 1982). Predation is not the only risk faced by *Exyra* within pitchers. Drowning is another risk. Phytotelm, water that accumulates within the pitcher, poses a serious problem to *Exyra* larvae (Rymal 1980). To reduce risk of drowning, *Exyra* larvae chew drainage holes below their roost. Within tall-form *Sarracenia*, *Exyra* often cover their pupation chamber with silk. Subsequently, they pupate within frass held by the leaf (Rymal and Folkerts 1982). Moth behavior is central to *Exyra* success in pitchers. Adults always face upward within pitchers, enabling them to avoid sliding downward because of downward pointing hairs and cellular projections in the conductive zone (Rymal 1980, Stephens and Folkerts 2012).

Acarids also use pitchers for shelter and food and occur in all *Sarracenia* species studied (Muma and Denmark 1967, Rymal and Folkerts 1982, Tagami 2004). For example, phytoseiids occur in many tall-form pitchers (Rymal and Folkerts 1982) and histiostomatids, *Sarraceniopus*, occur in most pitcher plants (Fashing and O'Connor 1984). *Sarraceniopus* comprises three species of interest. *Sarraceniopus gibsoni* occurs in Purple Pitcher Plants. *S. hughesi* occurs in Yellow Pitcher Plants, Sweet Pitcher Plants, Hooded Pitcher Plants and *Darlingtonia californica* Torr. (Cobra Lily) (Hunter and Hunter 1964). Finally, *Sarraceniopus nipponensis* Tagami was described from pitchers hybridized for horticulture in Japan (Tagami 2004). That *S. nipponensis* survived transition into horticultural varieties and trans-Pacific transplantations of *Sarracenia* spp. is an indication of their resilience to changes in the environment outside of their pitcher.

Pitchers provide acarids with a significant and stable resource of water, nutrition, and a safe site for reproduction. For example, *S. hughesi* is aquatic as an adult, but requires dry substrate for egg deposition and deutonymph maturation (Hunter and Hunter 1964). Both environments are provided in pitchers. Hunter and Hunter (1964) noted *S. gibsoni* only occurred in pitchers containing a prey mass. This observation was supported and extended by Trzcineski et al. (2003). Trzcineski et al. (2003) showed that abundance of *S. gibsoni* in dense patches of pitchers increases in presence with an increase in pitcher contents, while the dipterans *W. smithii* and *M. knabi* showed similar responses to pitcher contents at high and low densities of pitchers. Thus, pitchers have been shown to be a resource for their associates. However, the associates appear to respond differently to these resources (Trzcineski et al. 2003).

The difference in resource sensitivity between mites and other arthropods may be explained in terms of cost and benefit. Since mites require relatively less space and nutrients than both *W. smithii* and *M. knabi*, the cost of moving to a new patch is not offset by resource gain. In

essence, mites may survive within smaller pockets of resources than other arthropods, but they have to work harder to move between pockets of resources. This would explain the patterns of dense aggregations noted by Trzcineski et al. (2003).

Sarraceniopus are not the only mites that inhabit *Sarracenia*; the phytoseiid *M. biscutatus* is also found within tall-form pitchers. Phytoseiids are known for their free-living habit (i.e. they are not parasitic on insects) (Chant 1965). Phytoseiid mites include species with strategies along a generalist/specialist continuum. It is possible that the generalist feeding strategy is the ancestral strategy and the specialist strategy is the derived state (McMurtry and Croft 1997). Phytoseiids likely arose from the generalist feeding taxon, Ascidae. *Macroseius biscutatus* employ a generalist feeding strategy in that they can survive on multiple prey sources, such as pollen, nematodes and histiostomatids, as reported by Muma and Denmark (1967). *Macroseius biscutatus* is distinguished from other phytoseiid species by the equal division of its dorsal shield, a characteristic shared with other Otopheidomeniinae (a sub-family of Phytoseiidae).

The unique habitat in which *Sarracenia* and their inhabitants occur is fire maintained (Keddy et al. 2006). Prescribed burns are often used to maintain what remains of this habitat. Prescribed burns have a well documented effect on vegetation (Carter and Foster 2004). However, fire's impact is poorly understood with regard to insects and other arthropods. Arthropod populations within pyrogenic habitats experience severe population attrition immediately following fires (Swengel 2001, Vasconcelos et al. 2009). Nevertheless, highly mobile insects and arthropods are favored because they can quickly re-colonize burned areas (Swengel 2001), and some insect populations show a positive response to fire (Panzer 2002). A positive response to fire has not been documented in pitcher plant wetlands. Stephens and

Folkerts (2012) noted the possible extirpation of the relatively mobile pitcher plant moths, *Exyra* spp., from sites where the less mobile acarids persist (pers. observation). Swengel's concern about insect extirpation caused by fire is made more serious by the fragmented nature of the currently remaining pitcher plant wetlands. Stephens and Folkerts (2012) have already reported that some extirpations have occurred due to poorly timed burns; however, it is unclear why less mobile arthropods, such as mites still persist in these populations.

While mites are able to persist in fragmented populations (Stephens and Folkerts 2012) and within horticultural varieties of *Sarracenia* (Tagami 2004), larger arthropods, such as *E. semicrocea*, have already evidenced signs of decline (i.e. extirpations and genetic structuring) (Stephens et al. 2011). Genetic structuring of populations has been shown in *E. semicrocea* as well as other species of pitcher plant wetlands. Sheridan and Karowe (2000) showed that fragmented populations of Yellow Pitcher Plants experience inbreeding depression which has led to lower seed set and seed viability. Similar studies focused on Sweet Pitcher Plant and *S. jonesii* (Jones' Pitcher Plant) revealed low genetic diversity. Godt and Hamrick (1998) declared that Jones' Pitcher Plant is at risk of genetic erosion while the same fate may be avoided for the Sweet Pitcher Plant if the populations are well managed.

Both pitcher plants and their associates are affected by fragmentation of their habitat. Therefore, it is imperative to manage for both pitcher plants and their inhabitants to maintain the communities in which these unique relationships occur. Although fire management is good for the plants of pitcher plant wetlands, insect extirpation due to fire is likely a concern for many pitcher inhabitants such as *Exyra* (Stephens and Folkerts 2012). In the past, it is likely that recolonization of a burned bog occurred from refugia in unburned patches or from an adjacent unburned bog. In small bog remnants, forest managers try to maintain declining pitcher plant

populations through prescribed burns (Carter and Foster 2004). However, the small size of the bogs combined with the distance between bog remnants may result in lack of refugia for bog inhabitants during fire, lack of nearby populations for recolonization, and thus may result in population extirpation. However, establishment of more holistic management practices requires more detailed information about the ecology of pitcher plant associates and how they respond to frequent and intensely managed fire.

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Chapter 2: Observations On The Ecology And Behavior Of *Macroseius biscutatus*, An Obligate Pitcher Plant Mite

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Abstract:

Habitat for tall-form pitcher plants of the genus *Sarracenia* and their associates is disappearing from the southeastern United States. *Macroseius biscutatus*, a pitcher dwelling Phytoseiid, and other arthropod associates of pitcher plants may have suffered decline in response to habitat fragmentation and fire management. Surveys were performed at sites in Alabama and Florida to determine the presence or absence of arthropod associates. Mite abundance was compared among bog zones: shrub, center and edge. Mite position in pitchers as well as temperature and humidity within and outside of pitchers were recorded for a 24 hour period. Behavioral experiments were performed to observe mite response to smoke. Surveys showed that *Exyra semicrocea* has been extirpated at some sites but that *M. biscutatus* continues to persist. *M. biscutatus* is more likely to occur in pitchers without *Exyra* frass than in pitchers with frass. Daily humidity changes are buffered within pitchers but mite position in pitchers does not show an obvious pattern. Experiments showed *M. biscutatus* increases movement in response to smoke

produced via combustion of *Pinus palustris* (Longleaf Pine) needles but not in response to other stimuli (steam, air). This response to smoke may provide an explanation for the persistence of *M. biscutatus* at sites where *E. semicrocea* and potentially other pitcher inhabitants have experienced extirpation. Movement in response to smoke may allow *M. biscutatus* to find refuge from approaching fires.

Introduction

Carnivorous plant habitats in the southeastern United States contain a unique richness of biodiversity. Over 29 species of carnivorous plants occur within this region (Stephens and Folkerts 2012), and many of them occur within pitcher plant wetlands. Pitcher plant wetlands are found within Longleaf Pine forests and range from upland locations to pocosins and wet pine savannahs (Folkerts 1999). The genus *Sarracenia* includes several tall-form species, e.g., *Sarracenia leucophylla* L. (White Topped Pitcher Plant), *S. flava* L. (Yellow Pitcher Plant), *S. rubra* Walt. (Sweet Pitcher Plant), *S. oreophila* (Kearney) Wherry (Green Pitcher Plant) and *S. alabamensis* Case and R.B. Case (Canebrake Pitcher Plant) as well as the phytotelm bearing *S. purpurea* L. (Purple Pitcher Plant) and the prostrate *S. psittacina* Michx. (Parrot Pitcher Plant) (Folkerts 1999).

There are more than 17 species of arthropods associated with pitcher plants, many of which have adaptations allowing them to circumvent the trapping mechanisms of these carnivorous plants and inhabit pitchers or otherwise engage in some level of symbiosis with pitcher plants. Pitcher plant associates include *Exyra* spp. (Noctuidae) (Atwater, et al. 2006, Jones 1921, Moon, et al. 2008, Stephens and Folkerts 2012), *Fletcherimyia* spp. (Sarcophagidae) (Dahlem and Naczi 2006), *Isodontia mexicana* Saussure (Sphecidae) (Folkerts 1999),

Sarraceniopus spp. (Histiostomatidae) (Fashing and O'Connor 1984, Kruger and Wirth 2011, Tagami 2004), and *Macroseius biscutatus* Chant Denmark and Baker (Phytoseiidae) (Chant et al. 1959, Muma and Denmark 1967) as well as several others.

Pitcher plant habitats are declining due to anthropogenic development and the disruption of fire regimes (Jennings and Rohr 2011). As a result, pitcher plants and their unique associates are disappearing from the southeastern United States (Jennings and Rohr 2011, Stephens and Folkerts 2012). Stephens and Folkerts (2012) reported that *Exyra semicrocea* Guenée has been extirpated from several small and isolated pitcher plant sites and suggested that prescribed burns and their timing at these sites are responsible. Furthermore, pitcher plant wetlands in which pitcher plant moths and other arthropod associates persist are usually large and heterogeneous with sub-habitat zones including areas dominated by grasses or by shrubby vegetation (pers. obs.).

Pitcher plant wetlands are host to plant species that are tolerant of fire and frequent fire is necessary to maintain diversity and stability of these habitats (Keddy et al. 2006). It is not clear how pitcher plant associates respond to fire, but it can be assumed that some if not all of them are also adapted to be fire tolerant. Many studies have shown that arthropods in pyrogenic habitats decrease in abundance after fire but return to near pre-fire population levels with relative rapidity (Vasconcelos et al. 2009). Relatively rapid recovery rates of arthropods as compared to larger animals may result from shorter life cycles and smaller resource requirements for survival, allowing arthropods to effectively use resource patches in burned habitat (Engstrom 2010). This size-related trend likely holds true even within arthropods, since smaller arthropods require fewer resources than larger arthropods. As a possible illustration of this, histiostomatid pitcher plant mites native to the southeastern United States have been reported from pitcher plants

cultivated in Japan (Tagami 2004). The work by Tagami (2004) tangentially points out the ability of these small arthropod associates to survive with relatively little resources.

Patchy burns may offer small arthropods potential resources in the form of refuge from fire. For example, in pyrogenic grassland habitats, survival of *Papaipema silphii* Bird (Silphium Borer Moth) populations seems to be dependent on the patchiness of the fire (Andrew and Leach 2006). For pitcher plant associates, heterogeneity in pitcher plant wetlands may result in fire patchiness and therefore in potential refuge from fire. Heterogeneity of the vegetation often results from differences in the underlying hydrology (Segelquist et al. 1990) and includes areas where fires will burn with different intensities. In addition, there is variation within pitchers, which can be subdivided into attractive, conductive and detentive zones (Juniper et al. 1989). The detentive zone in tall-form pitchers commonly contains a moist prey mass (Glenn and Bodri 2012) and may provide a refugium for mites during fire.

I present a three tiered study to address how mites respond at the levels of habitat, microhabitat and behavior. Habitat level investigation comprised presence/absence surveys to determine if there was variation in pitcher inhabitants among sites and (in the second year) among sub-habitat zones. Microhabitat level investigation included recording of pitcher environmental condition to determine if the microclimate within the lumen of the pitcher varies from the external environment. Additional observations during the same 24 hour period included mite position within pitchers. Behavioral observations were made of mite position in pitchers in the lab using a growth chamber to vary relative humidity and temperature. Also in the lab, mite response to changes in relative humidity and smoke produced by Longleaf Pine needles was measured, to determine if *M. biscutatus* responds to cues indicating fire.

Site Descriptions:

Surveys were carried out at nine localities on public land and private land with Nature Conservancy easement (Fig. 1). These include one site with Green Pitcher Plants in Cherokee County (CC) and three with Canebrake Pitcher Plants: one in Autauga County (TK), and two in Chilton County (MM, RC). The remaining five sites contained White Topped Pitcher Plants, Sweet Pitcher Plants and Yellow Pitcher Plants and were in Baldwin Co. AL (SH) and (BB), Covington Co. AL (CU) and (CR), and Liberty Co. AL (SA).

Study Species

Macroseius biscutatus Chant, Denmark and Baker (1959) is a member of the Phytoseiidae, a family which possesses distinct palpal claws and dorsal shields. The dorsal shield is bisected, as indicated by the specific epithet. In spite of being able to survive on multiple food sources, it is only found with regularity in pitchers of *Sarracenia* spp. (Muma and Denmark 1967). Furthermore, it is the only species of phytoseiid known from pitcher plants (Adlassnig et al. 2011). Because its size is notably larger than other Acari known from pitcher plants (see Tagami (2004) for representative lengths of histiostomatids) it is easily recognized in the field and without magnification. Size was the key characteristic I used when sorting Acari from samples.

Methods

Macroseius biscutatus identification in samples

Macroseius biscutatus individuals were initially identified by size and counted by eye or through a dissecting microscope. To ascertain the accuracy of this method, a subset ($n = 18$) of

arbitrarily selected mites were slide-mounted with Hoyers medium, and identified using dorsal setal arrangement and the bisected scutum as diagnostic features (Chant et al. 1959).

Habitat Surveys

Habitat surveys before 23 May 2012 were general in nature and established the types of associates present at each site. These habitat surveys were later augmented by more detailed microhabitat surveys during 23 May 2012 to 25 August 2012. Pitchers were collected arbitrarily along belt transects at each site: MM ($n = 12$), RC ($n = 21$), TK ($n = 23$), CU ($n = 24$), CR ($n = 28$), SH ($n = 59$), BB ($n = 72$) and SA ($n = 57$) (Fig.1). At each site, pitcher plant wetlands were divided into three sub-habitat zones: shrubby, center and edge. Shrubby zones contained dense woody growth a meter or more in height. Center zones comprised large areas dominated by grasses. Edge zones were considered as the ecotone between the shrubby zone and center zone of each transect where the shrubby vegetation became patchy and intermixed with grasses. Transects were positioned so that one end of each transect was anchored in an area with pitcher plants in a shrubby zone and the other end was anchored in the approximate center of a grassy zone. Pitchers were collected along transects and in equal proportions from each zone, except in one case when pitchers could not be located in the shrubby zone for RC. For each pitcher, I recorded site, date of collection, position in the transect, species of pitcher plant, type and number of pitcher plant associates, pitcher aperture circumference, and depth of pitcher lumen. Pitchers were grouped as early or late based on date of collection. Pitchers collected between May and June were considered as early, those collected between July and August were considered late. The time in months since the last burn was recorded when known or was estimated for each site. Each site was assigned to either the northern region (Autauga, Cherokee, and Chilton counties) or southern region (Baldwin, Conecuh, and Liberty counties). Pitcher plant

associates could often be detected visually in the field, but pitcher contents were also transferred to filter paper and subjected to extraction with Berlese funnels for 48 hours. Associates were counted under a dissecting microscope. After Berlese extraction, pitcher contents were removed from the filter paper, dried in a drying oven for 48 hours, and weighed.

I used a generalized linear model with random effects (GLMER) to determine which variables best predicted phytoseiid abundance. A GLMER with a Poisson distribution was constructed in which phytoseiid abundance was analyzed as a function of time since burn, volume of pitcher (calculated using pitcher aperture and lumen depth), presence of other pitcher plant associates, dry mass of pitcher contents, sub-habitat zone, early season vs. late season, an interaction for time since burn and zone, and a random effect for transect to control for variability within and between sites. Calculations were carried out in R v 2.15.0 (package lme4) (R Development Core Team 2012).

Microhabitat Studies

Investigation at the microhabitat scale included measurements of relative humidity and temperature within pitchers as well as observations of mite position within pitchers during a 24 hour period. I measured the relative humidity inside pitchers and outside within 0.3 meters of each pitcher and 0.3 meters from the ground every 3 hours from 15:00 to 12:00 from 1 August 2012 to 2 August 2012 at a site in Baldwin county, AL. Twenty pitchers were sampled at each time interval ($n = 160$). To determine if conditions varied as a function of time, these data were assessed using an Analysis of variance (ANOVA), and if ANOVA was significant a Tukey's HSD post hoc test was executed in R v. 2.15.0 (R Development Core Team 2012).

To determine how mites respond to variation in pitcher microclimate, ten undisturbed pitchers were arbitrarily selected at each time interval and slit open for observation. Mite number, position, pitcher condition, presence versus absence of insect damage, presence and type of associates, and type of subhabitat from which pitcher was collected were recorded for each pitcher. Only mite-containing pitchers were used in analysis ($n=35$). First I constructed a full linear model and used maximum likelihood to fit the model, as opposed to restricted maximum likelihood. Response variable for mite position was created by subtracting the number of mites in the detentive zone from the number of mites in the conductive zone. Response variable was set as a function of zone, pitcher condition, pitcher plant associate, frass, time of day, average temperature obtained from microclimate observations, average relative humidity obtained from microclimate observations, and presence vs absence of insect damage. I then created a reduced model by omitting parameters that lacked significant variation and compared it to the full model. Reduced models were kept if the omission of a parameter did not significantly ($p < 0.05$) affect model fit under full model comparison (Murtaugh 2009). Statistics were calculated using R v. 2.15.0 (R Development Core Team 2012).

Behavioral Studies

In order to separate the effects of humidity and temperature on mite position within pitchers, I performed a manipulation in the laboratory. A minimum of ten mites were added to each of 27 open pitchers held upright in cups. Pitchers with mites were placed in a growth chamber and mite positions were observed at a variety of temperature and humidity settings. To score mite position, the difference between numbers of mites in the detentive zone and the conductive zone was calculated. Some mites escaped during the experiments. To control for the resulting variation in sample size, each mite was counted as the fraction of the total number of

mites detected for that treatment. For example, if a hypothetical total of 20 mites were detected for a treatment but only 1 mite was detected in 1 pitcher, this mite would be recorded as +0.05. This convention keeps small numbers of mites from exerting an undue influence. These data were analyzed using a linear mixed effects model (LMER) in R v. 2.15.0. (package lme4) (R Development Core Team 2012). First I constructed a full LMER and used maximum likelihood to fit the model, as opposed to restricted maximum likelihood. I then created reduced models and compared them to the full model. Reduced models were kept if the omission of a parameter did not significantly ($p < 0.05$) affect model fit under full model comparison (Murtaugh 2009).

To determine if mites respond to humidity gradients, temperature gradients or to smoke, mites were exposed to a variety of conditions within a specially designed chamber. Their response was measured as a change in position on a wire suspended in the chamber. The chamber consisted of a glass cylinder (15 cm X 45 cm, standing vertically, open on the lower end, and closed at the top) within which a 45 cm length of 0.17 cm diameter steel wire was suspended from a wooden disk held within the chamber at the closed end. Three wooden legs fit inside the cylinder, supported the wooden disk and wire, and were fixed to a square wooden box underneath. The top of the box supported the cylinder and was constructed with a circular hole in the center and beneath the suspended wire. The front of the box was open. The wire was marked at 5 cm intervals.

Temperature and relative humidity within the chamber were measured using a probe fixed to one of the wooden legs. Mite position, temperature, and relative humidity within the chamber were measured every thirty seconds during observation of mites (Fig. 2). Mites used in this experiment were collected from the field ($n = 62$).

To create a gradient of humidity, a bag of Drierite© desiccant was suspended from within the top of the chamber and heated water was placed in a glass Pyrex© dish beneath the aperture so that it covered the aperture completely (9 mites, 99 total observations). Mites were started at either high (30,35 or 40cm) or low positions (5cm). Additional trials involved desiccant and no water (20 mites, 214 total observations) and neither desiccant nor water (19 mites, 203 total observations). Finally, for the smoke treatment, smoldering *Pinus palustris* Mill (Longleaf Pine) needles were positioned beneath the aperture and the open side of the box was covered with a Plexiglas shield. To assess the effect of the treatment on the vertical position of mites on the wire (12 mites, 128 total observations), treatments were compared using a LMER model in R v. 2.15.0 (package lme4) (R Development Core Team 2012).

To determine if mites respond to smoke when inside pitchers, mites in pitchers were subjected to smoke and other treatments in another specially constructed chamber. This chamber consisted of a 1 X 0.5 X 0.5 m wooden frame constructed covered with 4 mil painter's plastic. A dryer vent, 7.6 cm in diameter and 2.42 m in length, extended from a fire chamber (a clean 3.8 liter paint can sealed with duct tape) to the inside of the experimental chamber and was attached to a support on the frame so that it was suspended from the top of the chamber (Fig. 4). A probe was positioned so that the sensor was inside the vent to measure maximum heat and relative humidity (arrow, Fig. 3). The probe was moved for 2 treatments during which it was supported in the chamber at approximately the height of the pitchers. This step was taken to keep water from condensing on the probe. Each of ten White Topped Pitcher Plant pitchers was loaded with 10 *M. biscutatus*. Pitchers and *M. biscutatus* were collected from the Splinter Hill Complex on 17 November 2012. Pitchers were supported in flower arranging foam inserted into 532 ml cups filled with water (Fig. 3). Pitchers containing mites ($n = 20$) were exposed to a series of

treatments. After each treatment, if any mites left the pitcher, they were returned to the lumen of the pitcher before the next treatment was applied. Treatments consisted of un-manipulated air within the box, steam, and smoke produced by Longleaf Pine needles. Treatments were applied in a series beginning with air, after which smoke and steam categories were alternated twice. The final treatment was always a smoke treatment. After the final treatment, pitchers were slit open and the mites remaining inside were counted. To determine if treatments affected the rate at which mites emerged from pitchers, and to determine if the number of mites leaving a pitcher was significantly different from those remaining in the pitcher, these data were assessed using a LMER in R v.2.15.0 (package lme4) (R Development Core Team 2012). Pitcher was set as a random effect.

Results

***Macroseius biscutatus* identification in samples**

Examination of cleared mite specimens via light microscopy revealed that our method of identifying *M. biscutatus* by size was relatively accurate. Ninety-four percent of specimens were correctly identified. Furthermore, two large mites collected from pitchers and identified by eye as not *M. biscutatus* were indeed different mites, one a bdellid and one an oribatid.

Habitat Surveys

No *Exyra* were detected at the sites in Autauga, Cherokee or Chilton counties (RC, MM, TK, CC). Sarcophagids were not detected at the sites in Chilton or Center counties (CC, MM) nor at one of the two Autauga County sites (TK), and *Isodontia* were not detected in Liberty County (SA). However, histiostomatids and phytoseiids were present at all sites. The results of the habitat surveys are presented in Table 1.

The results of the microhabitat survey from May 2012 to August 2012 are presented in Table 2. The microhabitat survey showed *M. biscutatus* presence was negatively correlated with the presence of *Exyra* frass (Table 2, Fig. 5). Mite presence was also negatively correlated with the dry mass of pitcher contents. Mite presence was positively correlated with the presence of sarcophagid larvae (Table 2, Fig. 6) and sciarid flies (Table 2, Fig. 7). Late season pitchers contained more mites than early season pitchers (Table 2). Pitchers collected at the southern sites contained fewer mites (Table 2).

Microhabitat Studies

Measurements of pitcher microclimate showed that pitcher leaves remain more humid than the surrounding environment (Fig. 8) during the hotter and drier parts of the day and that the physical condition of pitchers may influence pitcher microclimate. There was no significant difference in temperature between pitcher lumen and environment at any time of day (Table 3). The reduced model showed that mite changes in position were positively correlated with average temperature (Table 4) and negatively correlated with the time of day (Table 4).

Behavioral Studies

In pitchers placed in the growth chamber, mites showed no response to humidity in chamber treatments: thus humidity was excluded from the reduced model (Table 5). Mite position changed with temperature (Table 5).

In the experiment in which *M. biscutatus* were placed on a wire, mites showed a significant response to smoke but no significant response to changes in relative humidity. *M. biscutatus* wire position was lower (8.2 ± 3.4 cm, $p = 0.019$; Table 6) when exposed to smoke. Additionally, these tests show that mites have a downward tendency in their movement. Mites

moved down 0.49cm (± 0.073 cm, $p < 0.001$; Table 6) for every 1cm in height of their starting positions.

In the experiment in which mites in pitchers were exposed to air, smoke and steam, *M. biscutatus* showed a significant response to smoke but not to steam or air. On average, more mites (5.6 mites ± 0.14 , $p < 0.0001$; Fig. 9) left per pitcher in the smoke treatment, while no mites left when treated with air or steam. On leaving the pitcher, mites moved down and were found at the base of the pitcher above or at the level of the water surrounding the leaf. There was no significant difference between the number of mites on the inside and outside of each pitcher per smoke treatment. There were an average of 1.7 (± 0.89 , $p = 0.061$) more *M. biscutatus* on the outside than on the inside. Finally, 3 *E. semicrocea* larvae were discovered in 3 treated pitchers. While larval behavior within pitchers went unobserved during treatments, it is clear that *E. semicrocea* did not exit pitchers as a result of the treatments.

Discussion

Habitat Surveys

The failure to detect *Exyra*, sarcophagid and *Isodontia* at several sites creates concern. *Exyra* were not detected at any of the northern sites. These small isolated habitats may be a challenge for *Exyra* especially since *Exyra* larvae are particularly vulnerable to winter burns, a common management technique (Stephens and Folkerts 2012). Sarcophagids were not detected at two of the northern sites (MM, TK) but were detected nearby at RC. Failure to detect sarcophagids at MM and TK is a concern but may not indicate extirpation. Finally, the failure to detect *Isodontia* in SA is not a concern since it is a relatively rare pitcher plant associate, and my survey was not exhaustive.

There may be scientific explanations for the correlations between pitcher plant associates observed in the microhabitat survey. Muma and Denmark (1967) showed that *M. biscutatus* did not mature or reproduce on *Exyra* frass nor did it reproduce on insect remains. Parameters of *Exyra* frass and dry mass of pitcher contents were correlated with a decrease in *M. biscutatus* abundance in this study. Importantly, these parameters have been identified as non-optimal food sources in the study by Muma and Denmark (1967). Thus, the presence and/or abundance of prey or frass may inhibit mites in accessing an optimal food source, since nematodes and histiostomatids are found inside the prey mass (pers. obs.). Second, dipteran, sarcophagid and sciarid larvae were associated with increased abundance of *M. biscutatus*. This may indicate that dipteran larvae and *M. biscutatus* have similar habitat requirements. Other possibilities include dipteran larvae serving as microhabitat modifiers or as phoretic hosts. As microhabitat modifiers, dipteran larvae may increase the availability of food for *M. biscutatus* through changes these larvae create in the prey mass. As phoretic hosts, adult dipterans may provide a mode of transportation between pitchers for *M. biscutatus* since dipterans must land on pitchers to larviposit. *M. biscutatus* has no known phoretic hosts. However, these conclusions are provisional, since the number of samples containing dipterans composed a very small percentage of the total (i.e. less than 2% for sciarid larvae and 22% for sarcophagid larvae). Thus, the relationship between dipterans, particularly sciarid larvae, and *M. biscutatus* may be a statistical artifact. More investigation is needed and future research should focus on clarifying the relationship between dipterans and *M. biscutatus*.

Microhabitat Studies

In my measurements of the diel variation of relative humidity within pitchers, I showed that the lumen of the pitcher remains more humid than the surrounding ambient air (Fig. 8). The

stability of microclimate, including temperature and humidity, within pitcher leaves has been previously suggested by Folkerts (1999). However, my analysis does not show a significant difference in temperature between pitchers and their surrounding environment (Table 3). The higher relative humidity within pitchers may be a benefit pitchers provide to their associates. The microclimate of the pitcher lumen may be more suitable for egg hatch and larval survival than the surrounding environment. For example, Croft et al. (1993) showed that phytoseiid egg and larval mortality is increased at low levels of relative humidity. Thus, a humid pitcher lumen likely provides an environment with lower rates of mortality for *M. biscutatus* than the surrounding vegetation.

Behavioral Studies

Mite behavior in pitchers was complex. The two most significant parameters, temperature and time of day counteracted one another, and the R^2 (Table 4) was very low, indicating poor model fit. However, my results are in concert with an earlier study by Mori and Chant (1966) which suggested that relative humidity does not detectably influence phytoseiid behavior. Changes in position may instead be linked to feeding behaviors as Muma and Denmark (1968) observed that *M. biscutatus* dispersed within the pitcher to feed.

Lab observations of mites in pitchers yielded similarly convoluted results. Mites exhibited variation in behavior in and on pitchers, but without a clear pattern (Table 5). For example, mite position on pitchers was similar for both very low temperatures (2-5 °C) and hot temperatures (26-34 °C), but slightly hotter temperatures (36 °C) produced a significantly different result. It is possible that mites were responding to unmeasured parameters in addition to

temperature changes. Future study will be needed to resolve mite response to temperature and humidity.

Experiments comparing mite response to temperature, relative humidity and smoke demonstrated that mites moved down on the suspended wire when exposed to smoke and moved down in response to smoke when outside pitchers (Table 6). When observed, the movements of mites while leaving pitchers appeared to be in a straight line moving downward. However, downward movement was not a universal response as some mites moved upward. For most mites in pitchers, exiting a pitcher requires some upward movement. Nevertheless, the behavior of moving in response to smoke may contribute to *M. biscutatus* survival in a pyrogenic habitat since it may allow these mites to seek shelter ahead of a fire front.

However, survival of *M. biscutatus* within pitchers during fire may be variable. Thus, the behavioral response of leaving a pitcher in the presence of smoke may or may not be advantageous. By comparison, populations of Silphium Borer Moths, which mature in the stems of *Silphium* spp. of short grass prairies, are unaffected by fire (Andrew and Leach 2006). Nevertheless, it is evident from the work of many authors (Engstrom 2010, Swengel 2001, Vasconcelos et al. 2009) that fire does affect arthropod and mite abundance. Since *M. biscutatus* exhibited a distinct yet somewhat variable behavior in response to a smoke stimulus, and considering that success in avoiding fire is condition dependent, it seems that movement, however variable in direction, may carry a selective advantage for *M. biscutatus* in these pyrogenic habitats. Future work comparing behaviors of pitcher plant associates to fire may prove interesting.

Conservation Implications

Habitats of declining pitcher plants that are managed for plant conservation are burned as often and completely as allowable. Although some agencies are moving toward growing-season burns, most prescribed fires are and have been applied during the winter. *Exyra* caterpillars are most vulnerable during the winter and are not likely to survive such fires. In addition, isolated bog remnants are not likely to be re-colonized because of the great distance from other suitable habitat (Stephens and Folkerts 2012). However, it appears that the phytoseiid mite, *M. biscutatus*, persists in these habitats. My data help to explain the mite's ability to survive under conditions of fire.

Movement in response to smoke and the acarid's small size relative to other pitcher inhabitants represent plausible explanations for mite persistence in habitats from which pitcher plant moths have been extirpated. First, a fleeing response to smoke is present in mites and likely is absent or ineffective for *Exyra* larvae. This fleeing response may allow a mite to actively seek refuge from a fire front before it reaches a pitcher containing *M. biscutatus*. Such a response may be, in part, responsible for the difference in persistence between moth and mite. Second, mites are able to survive on minimal resources, as indicated in a study by Schneider et al. (2007) showing that Acari populations sustained little change even when isolated and confined to small areas of habitat less than 7 cm in diameter and 15 cm in length. This is probably true of *M. biscutatus* since it has the ability to survive on a variety of prey items (Muma and Denmark 1967). Mites that survive first order fire effects are not likely to suffer subsequent starvation, a common danger for other animals after fires (Engstrom 2010, Reinhardt et al. 2001).

In conclusion, *M. biscutatus* is a pitcher plant associate that persists in small, isolated habitat patches where other associates may have suffered extirpation. The persistence of *M. biscutatus* may be attributable to its increased movement in response to smoke, a precursor to

fire. This response may facilitate escape to refugia for *M. biscutatus* individuals. Future work on pitcher plant associates considering meta-population dynamics for *M. biscutatus* and *Exyra* sp. would provide further insight into the extirpation or persistence of these unique pitcher plant associates under current conservation regimes.

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Figures and Tables

Legend

Figure 1: Locations of study sites used for pitcher plant associate surveys. *Sarracenia* species present at each site are noted. Sites were surveyed for presence and absence of pitcher inhabitants and were visited between May 2011 and August 2012

Figure 2: Chamber for observation of *M. biscutatus* outside of pitchers. W = wire on which mites moved during treatment. PR = Temperature/humidity probe display. FT = Fire tray, small pie pans on which Longleaf Pine needles were burned

Figure 3: White Topped Pitcher Plant pitchers supported in cups inside the smoke chamber. S = support for drier vent. DV= drier vent through which smoke and steam were introduced to the chamber. P = Pitcher in which mites were placed. F = floral foam which supported the pitchers. PR = probe placement (probe was fixed to the inside of the drier vent).

Figure 4: Chamber for exposing mites to smoke while within pitchers. DV = dryer vent used for introducing steam/water vapor or Longleaf Pine smoke. B = smoke chamber to hold 10 pitchers in cups. EM = environmental manipulator used in the creation of water vapor/steam or smoke. HH = hand held monitor for viewing data collected by probe

Figure 5: Comparison of *M. biscutatus* presence and absence in pitchers with and without *Exyra frass*.

Figure 6: Mite presence as related to sarcophagid presence, for all sites ($n = 230$ for 0, $n = 52$ for 1 and $n = 5$ for 2)

Figure 7: *M. biscutatus* presence as related to presence of sciarid larvae ($n = 282$ for 0 sciarid larvae and $n = 5$ for 1).

Figure 8: Comparison of relative humidity in pitchers and outside within 0.3 m of pitcher during a 24 hour period. Letters which differ indicate a significant difference ($p < 0.05$) using Tukey's HSD in R version 2.15.0 ($n=20$ for all measures except for 0 $n = 21$ and for 3 $n = 19$).

Figure 9: Box plot of average number of mites leaving pitcher for each treatment. (20 pitchers, 88 mite observations). Different letters indicate significant difference ($p < 0.05$) calculated using LMER in R v. 2.15.0. Model was fit with restricted maximum likelihood function (AIC = 424.1958, BIC = 436.4091, logLik = -207.0979)

Table 1: Presence/Absence of pitcher inhabitants for each pitcher plant species. ND = not detected and P = present. (CC) = Central Bog, Cherokee County AL; (MM) = Miss Moore Bog, Chilton County AL, (TK) = Tukabatchee, Autauga County AL; (RC) = Roberta Case Nature Preserve, Chilton County AL; (BB) = Forever Wild Splinter Hill Complex, Baldwin County AL; (SH) = The Nature Conservancy Splinter Hill Bog, Baldwin County AL; (CU) = Curious Bog (or Parker Springs Bog), Covington County AL; (CR) = Crawford Bog, Covington County AL; (SA) = Sumatra Bog, Liberty County FL

Pitcher Plant Species and Sites	Phytoseiids	<i>Exyra</i>	Histiostomatids	Sarcophagids	<i>Isodontia</i>
<i>S. oreophila</i> (CC)	P	ND	P	ND	P
<i>S. rubra alabamensis</i> (MM, TK)	P	ND	P	ND	P
<i>S. rubra alabamensis</i> (RC)	P	ND	P	P	P
<i>S. leucophylla</i> and <i>S. flava</i> (BB, CU, SH, CR)	P	P	P	P	P
<i>S. leucophylla</i> & <i>S.</i> <i>flava</i> (SA)	P	P	P	P	ND

Table 2: Bog parameter estimates for GLMER with Poisson distribution. Model was fit using Laplace approximation. Estimates of likelihood have been interpreted using $e^{(\text{parameter})}$. Estimates are likelihood ratios thus 4.1 is interpreted as 4.1 times as likely. Random effect of transect (0.29 Variance \pm 0.54 SE). Finally, within categorical variables, the unlisted variable is the variable to which the other variables were compared and these variables were not significantly different from 1 for this model.

	Estimate	SD	T value	Pr(> z)
edge zone	0.86	1.2	-0.78	0.43
shrub zone	1.0	1.2	0.12	0.91
dry mass of pitcher contents	0.063*	3.7	-2.1	0.03
months since last burn	0.99	1.0	-0.59	0.56
histiostomatid	1.0	1.0	0.35	0.73
number of sciarid larvae	4.3***	1.4	4.0	6.2E-05
number of sarcophagid larvae	1.5***	1.1	3.5	0.00039
volume of pitcher lumen	1.0	1.0	0.042	0.97
<i>Exyra</i> frass absent	3.3***	1.3	4.6	4.0E-06
adult <i>Exyra</i>	1.1	1.5	0.19	0.85
late collection batch	2.4**	1.3	3.0	0.0024
south region	0.38*	1.5	-2.4	0.015
interaction between edge zone and months since burn	1.0	1.0	1.9	0.053
interaction between shrub zone and months since burn	1.0	1.0	0.62	0.54

Significance codes: (<0.001 ‘*’) (0.001 ‘**’) (0.01 ‘*)**

Model Fit (AIC = 877.9, BIC = 936.4, logLik = -422.9, deviance 845.9)

Table 3: Analysis of variance table for the difference in temperature between pitcher lumen and pitcher exterior as a function of time of day.

	Degrees of Freedom	Sum of Squares	Mean Square	F value	Pr(>F)
Time	7	0.338	0.04827	0.674	0.69
Residual	152	10.892	0.07166		

Table 4: Mite position in pitchers in pitcher plant habitat. Model is $Y = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3$

Parameters (X_n)	Estimate (β_n)	SD	T value	Pr(> t)
sarcophagid presence	-1.5	0.84	-1.8	0.087
average temperature °Celsius	0.78**	0.27	2.9	0.0072
Time of Day (9-15)	-5.1*	1.9	-2.6	0.014

Significance codes: (<0.001 ‘*’) (0.001 ‘**’) (0.01 ‘*’). Residual standard error: 3.463 on 31 degrees of freedom, Multiple R-squared: 0.27, Adjusted R-squared: 0.20, F-statistic: 3.9 on 3 and 31 DF, p-value: 0.01852.**

Table 5: Mite position in pitchers placed inside growth chamber at different temperatures. A reduced linear model with random effects was created using a stepwise regression. Analysis revealed that humidity did not affect mite position. For weighted average of mites a negative indicates more mites in the detentive zone than in the conductive zone and a positive indicates more mites in the conductive zone than in the detentive zone. Model is $Y = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3$. Random effect for plant (intercept=2.4, residual=5.4, observations =161, and mites=26). Model parameters that did not significantly affect model fit ($p < 0.05$) were dropped from the model (i.e. humidity).

	n=	Weighted average of mites in the conductive zone (β_n)	SD	DF	t-value	p-value
36 °C (reference)	n=27	5.2**	0.83	132	6.2	<0.001
2-5 °C and 26-34 °C	n=40	-6.1**	1.21	132	-5.0	<0.001
15-25 °C	n=61	-3.2**	1.2	132	-2.7	0.008

Significance codes: (<0.001 ‘*’) (0.001 ‘**’) (0.01 ‘*’) AIC 1030.876, BIC 1046.283, logLik -510.4379**

Table 6: Mite response to humidity and/or smoke. LMER was constructed using R ($Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 \dots \beta_j X_j$). Random effect for individual mite was accounted for (Intercept, residual = 9.236208, 5.444403). This model was fitted using restricted maximum likelihood. It contained 667 observations in 60 groups.

Parameter(X_n)	Estimate of effect (β_n)	SD	DF	T-value	p-value
(intercept) (X_0)	5.7 (β_0)	2.6	604	2.2	0.027
Desiccant	-1.1	3.5	56	-0.32	0.75
control Lab	-0.72	1.0	604	-0.36	0.72
control Hood	-1.1	3.6	56	-0.3	0.77
Smoke	-8.2*	3.4	56	-2.4	0.019
humidity gradient, water low desiccant high	0.92	2.0	604	0.46	0.65
height of starting postions	-0.49***	0.073	604	-6.7	< 0.001
Significance codes: (<0.001 ‘***’) (0.001 ‘**’) (0.01 ‘*’) AIC 4355.546, BIC 4395.976, logLik 2168.773					
-					

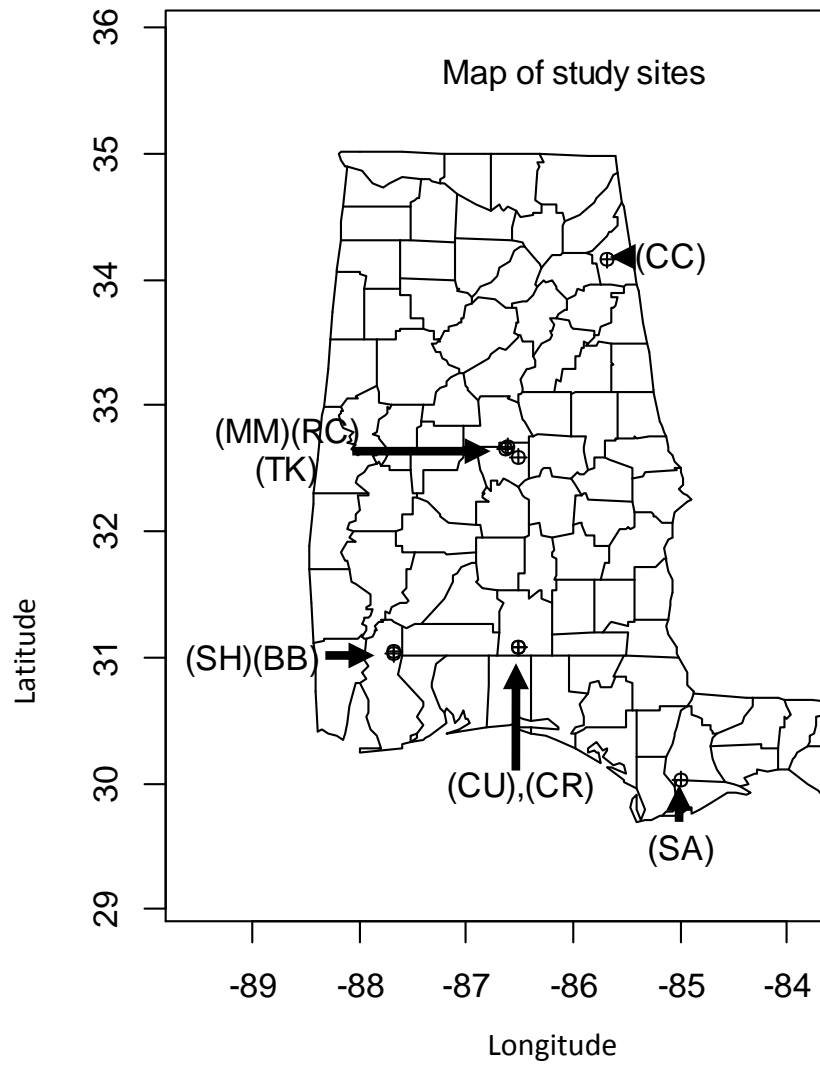


Figure 1

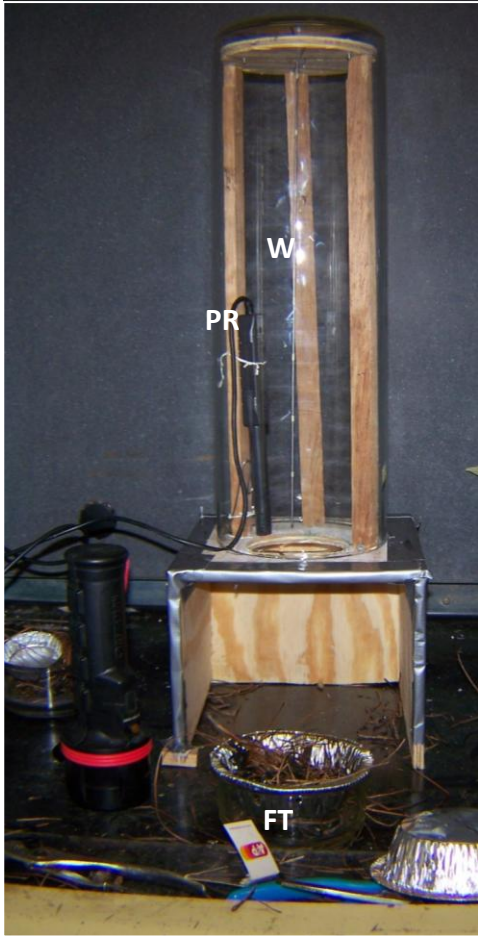


Figure 2



Figure 3



Figure 4

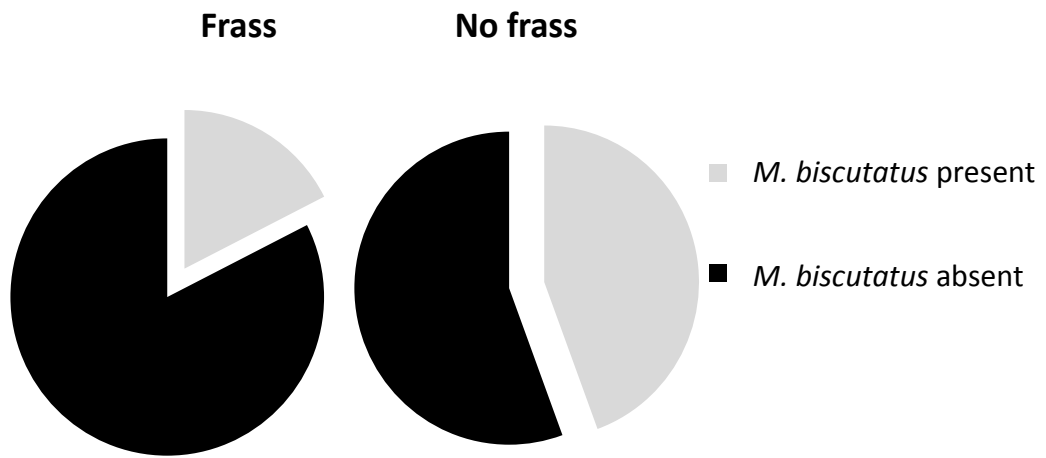


Figure 5

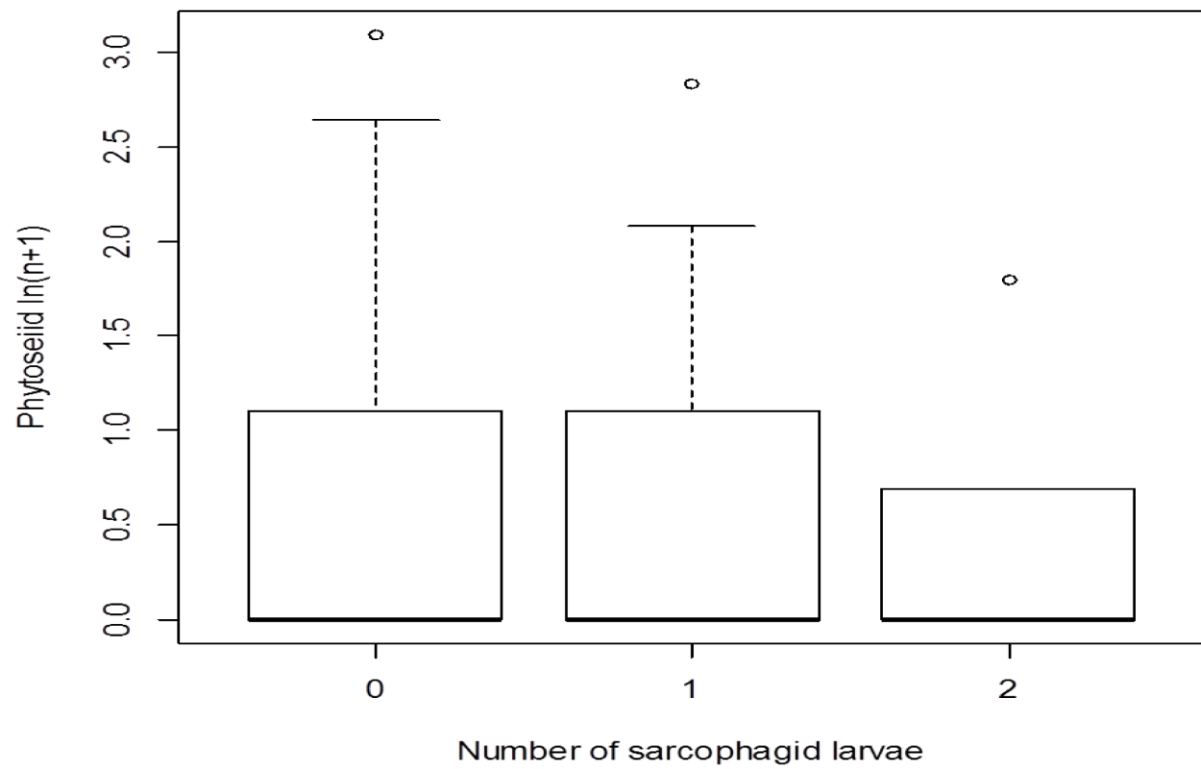


Figure 6

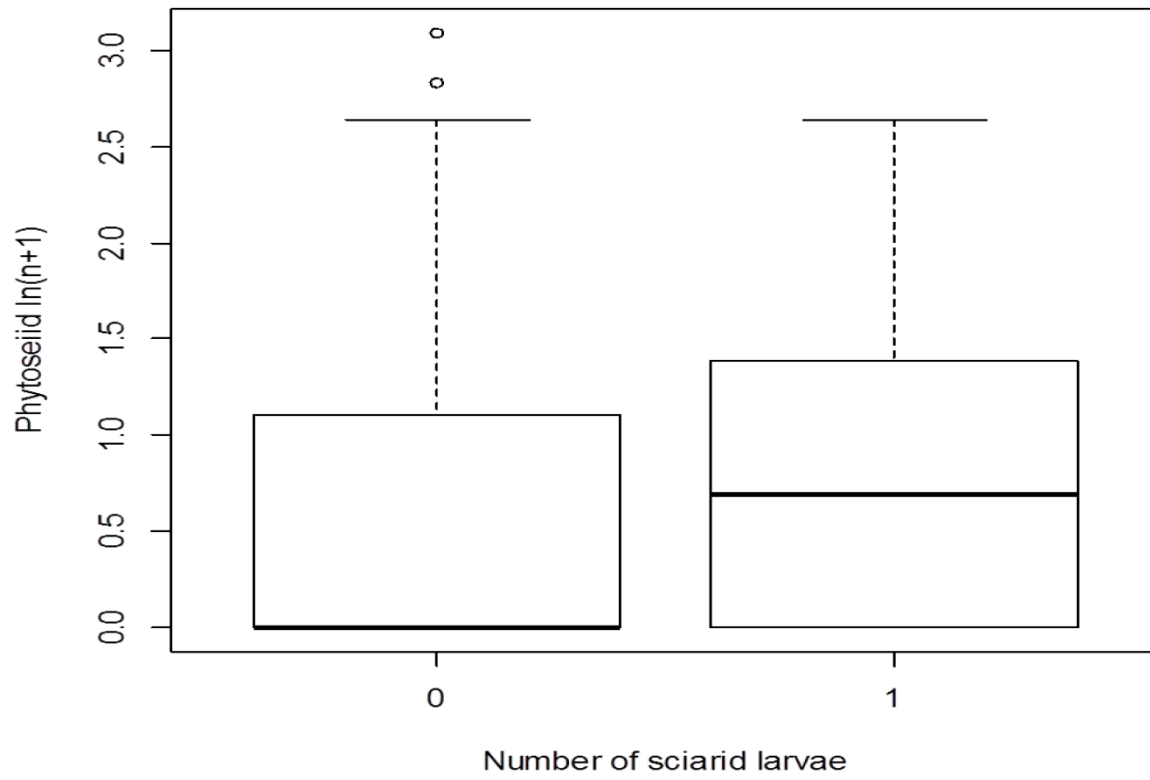


Figure 7

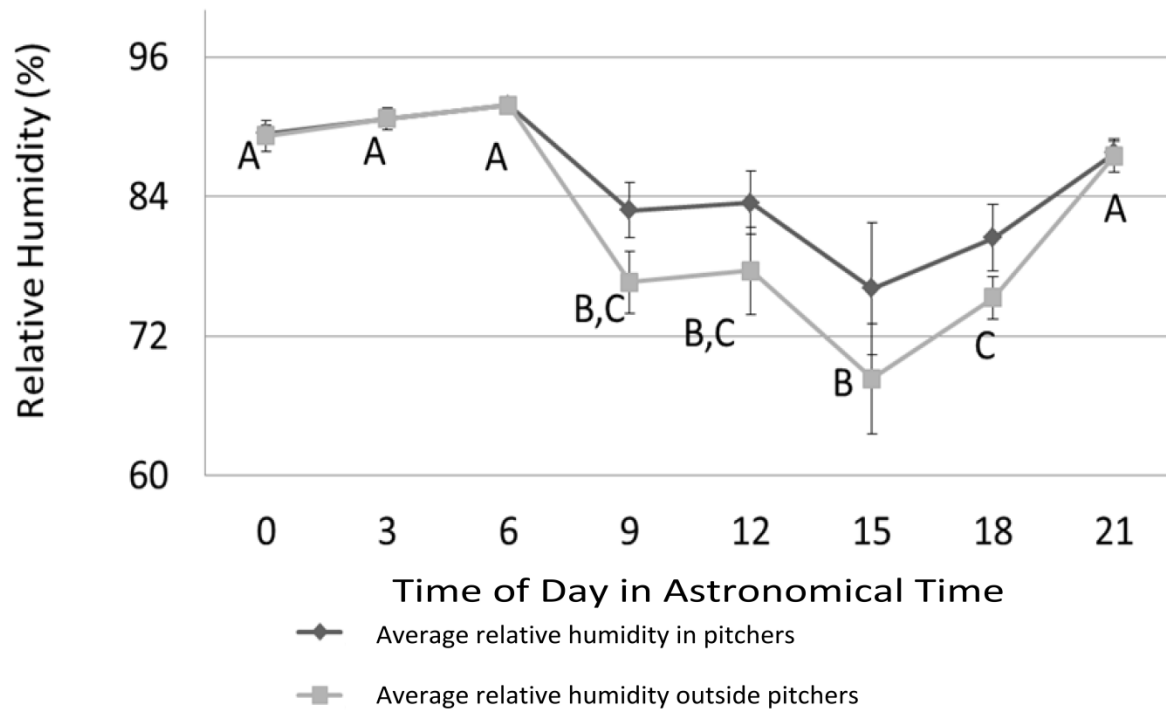


Figure 8:

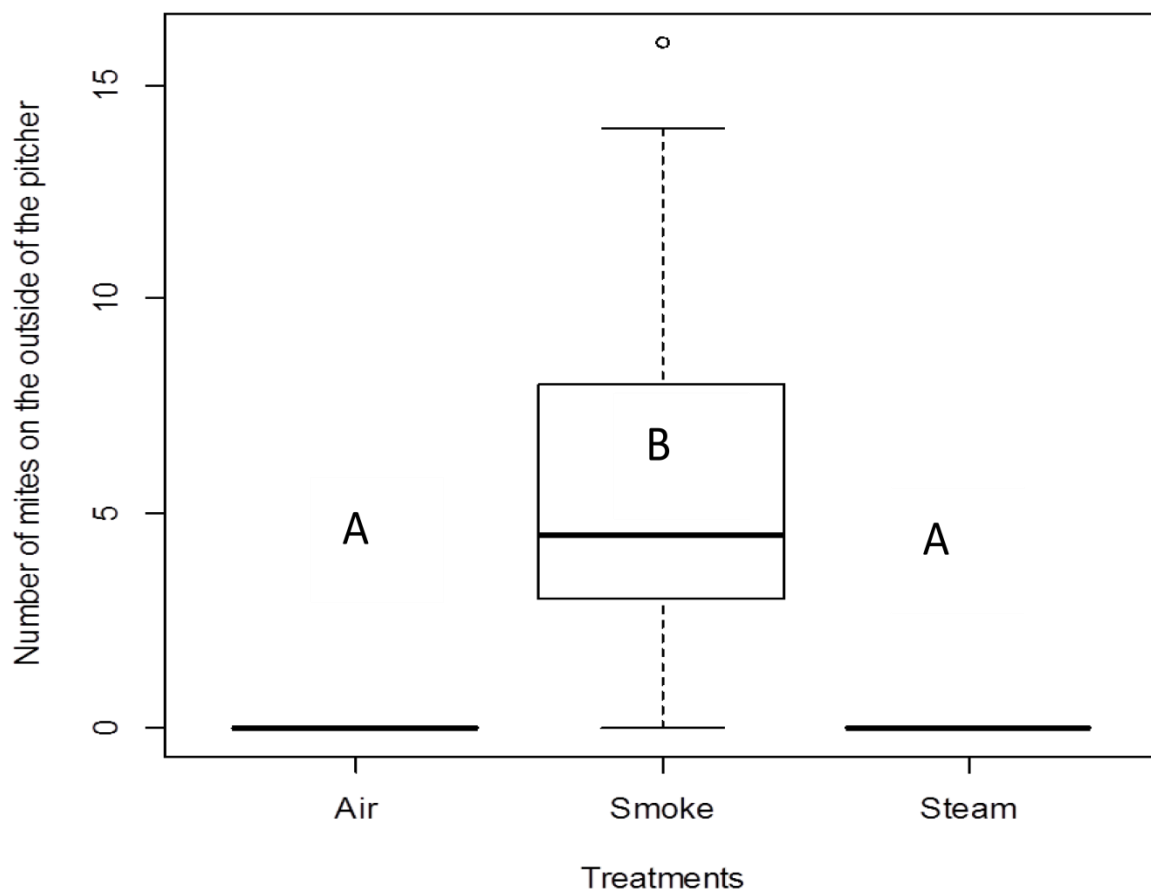


Figure 9: