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Seasonal Phenology, Distribution and Treatments for Polyphagotarsonemus latus (Banks) on Primocane-fruiting Blackberries (Rubus L. subgenus Rubus) in Arkansas

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Seasonal Phenology, Distribution and Treatments for *Polyphagotarsonemus latus* (Banks) on Primocane-fruiting Blackberries (*Rubus* L. subgenus *Rubus*) in Arkansas

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Abstract

Worldwide, blackberries (*Rubus* L. subgenus *Rubus*) are an economically important crop. In 2007, *Polyphagotarsonemus latus* (Banks) (broad mites), were first reported damaging primocane-fruiting blackberries in Fayetteville, Arkansas. Since this time, broad mite damage to blackberries and yield loss has been reported in many states and countries. Despite the increasing reports of this blackberry pest, little is known about their population dynamics, and few treatments are available. Growers with broad mite populations need a pest management program to minimize yield loss. Therefore, there was a need to develop sampling techniques, describe broad mite seasonal phenology in blackberry fields, and determine efficacy of various control tactics. The first step was to evaluate a modified two-step floatation water, sugar-water (WSW) method to extract broad mites from Tullgren funnel sample debris and compare counts across sample substrates. Then, broad mite seasonal densities were determined through biweekly sampling of leaves, leaf litter, and soil samples from a blackberry field in Arkansas. From November to late-February, *Polyphagotarsonemus latus* was found in highest numbers in the leaf litter. Sample counts also showed that the broad mite is highly aggregated prior to uniform distribution in the blackberry field (late May). Laboratory leaf-dip bioassays found that Agri-Mek caused 100% broad mite mortality after 24 hrs; after 72 hours, M-Pede, Microthiol Disperss, and JMS Stylet Oil caused >90% percent mortality and Quillaja caused 83% mortality. The results of these bioassays were used by the Agri-Mek manufacturer to label it for use in caneberries (blackberries) against broad mites in AR and several other states. Field tests were conducted to determine the efficacy of 4 different predatory mite species and sulfur (*Amblyseius andersoni* (Chant), *Neoseiulus californicus* (McGregor), *N. cucumeris* (Oudemans) and *N. swirskii* (Athias-Henriot). Individually, all four species of predatory mites had statistical differences from control plots in at least one field; species efficacy was different by location.

Data collected from the seasonal study, bioassays and field trials was used to develop a broad mite fact sheet describing the recommended integrated pest management program for broad mites on blackberries.

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Dedication

To my grandmother, Lois C. Brooks who wanted me to finish school and to my husband David, and children Joshua, Lois, Emma, and Lilian who supported me as I completed this degree.

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List of Published Papers

Chapter 2:
LeFors, J. A., D. T. Johnson, T. Kirkpatrick, T. Woodruff, and G. J. de Moraes. 2018. A two step centrifugation method with water and sucrose to separate mites from raw extracts of Tullgren funnels. Systematic & Applied Acarology 23(5): 000–000 (2018) ISSN 1362-1971 (print) http://doi.org/10.11158/saa.23.x.x ISSN 2056-6069 (online).
Systematic and Applied Acarology (Accepted).

Chapter 4:
LeFors, J. A., D. T. Johnson, and T. Woodruff. 2016. Acaricidal control of broad mites in blackberry, 2016. Arthropod Management Tests 42(1). tsx113, https://doi.org/10.1093/amt/tsx113.

Chapter 1: Literature Review on *Polyphagotarsonemus latus* (Banks) Taxonomy and Pest Management

The broad mite, *Polyphagotarsonemus latus* (Banks), is a worldwide pest to food and ornamental crops in 60 different plant families (Weintraub et al. 2003). In 2007, *P. latus* became a known pest of blackberry plants (*Rubus L.* subgenus *Rubus*). This pest continues to damage blackberries in many states and countries. However, there are few pest management tactics available against *P. latus* on these *Rubus sp.*, and nothing is known about their seasonal phenology in this crop. To develop an effective integrated pest management (IPM) program for *P. latus* on blackberry plants, new treatment methods and population dynamics need to be investigated.

Synonyms and Classification of *P. latus*

P. latus is known by several common names and since 1890, has been published with multiple scientific names. In the US, the *P. latus* common name is typically the broad mite, other used common names are the chilli mite; citrus silver mite; jute white mite; rubber leaf mite; tropical mite; and yellow tea mite (CABI 2018). Since 1890, literature has several published synonyms for *P. latus*: *Acarus translucens* Green, *Hemitarsonemus latus* Banks, *Tarsonemus translucens* Green, *Avrosita translucens* Oudemans, *T. latus* Banks, *T. phaseoli* Bondar, and *Neotarsonemus latus* Smiley (Lin et al. 2002). As of 2009, the current accepted name is *P. latus* Banks. *P. latus* is one of only two species in the *Polyphagotarsonemus* genera (the second species (*P. beeri* Smiley) has been reported only once in Africa in 1964) (Lindquist 1986; Vacante 2016). The broad mite is classified as follows: Superorder: Acariformes; Order Trombidiformes; Suborder: Prostigmata; Supercohort: Eleutherengonides; Cohort: Heterostigmatina; Superfamily: Tarsonemoidea; and Family: Tarsonemidae (Krantz and Walter

2009). The Tarsonemoidea family have a few distinct characteristics that are commonly used to classify its members.

Description of Superfamily and Family

The two main diagnostic characteristics of superfamily Tarsonemoidea include a stylet-like moveable digit formed from fused chelicerae with reduced nonarticulating palpi segments, and a small female genital opening. Additionally, female legs IV do not have claws or empodiums, the femur and genu are fused, and have 2-3 setae on the tibia and tarsus. Male legs IV have 4 segments, and a single or vestigial claw. The diagnostic characteristics for the family Tarsonemidae are slender female legs IV with three segments. Male legs IV are inserted ventrally and have 3-4 segments (Krantz and Walter 2009). *P. latus* has these characteristics and the following features that identify it to species.

Taxonomic description of *P. latus*

See Table 1 for definitions of taxonomic terms in this section. The main diagnostic feature of the adult female *P. latus* is the one distinctive claw on the end of leg I tibiotarsus (Lindquist 1986; Vacante 2016). The adult female *P. latus* has a gnathosoma capsule that does not have palpcoxal setae and is as wide as it is long. The dorsal gnathosoma setae are simple and smooth. Palpi are short, directed anteriorly and a bit medially, with single minute setae. Chelicera are stylets, with a slender pharynx, surrounded by well sclerotized walls. The pharynx is elongate and elliptically shaped with evident musculature. Often a small pair of glands are associated with the posterior of pharynx (Lindquist 1986). The adult female *P. latus* prodorsal shield does not cover the stigmata or the gnathosoma. *P. latus'* stigmata are close to v_1 (internal vertical setae on prodorsum). The external scapular setae (sc_2), is longer than any other setal tergital pair. Dorsal setae are short and slender. On plate EF, the setae e and f are transversely

aligned. Female broad mite ventral apodemes (1, 2, 3, 4, prosternal and sejugal) are distinct. Apodeme 1 forms a y shaped juncture and apodeme 2 is strongly connected with prosternal apodeme. The prosternal and sejugal apodeme are united and continuous. Apodeme 3 is unconnected to any other apodemes and extend slightly laterally of trochanters of leg III. Apodeme 4 is unconnected to other apodemes and extends posterolateral of setae 3b to bases of trochanter IV. The anterior margins of coxisternal plates are straight. On the metapodosomal venter there are 4 pairs of coxal setae. The base of leg IV is separated by an interval of 3x the width of trochanter IV (Lindquist 1986). Chaetotaxy of legs are distinctive for *P. latus*. The female lacks setae *l'* on femur I, lacks setae *pl*' on tarsus I, and the *pv'* (pretarsal setae) on tarsus I is enlarged and spinelike. On tarsus II, setae *pl*' is also spine like. On the tibiotarsus of leg IV, there are only two setae (Lindquist 1986).

There are two distinctive features of the adult male *P. latus*. Leg IV is long and the tibiotarsus is fused and elbow shaped with a reduced button-like claw (Lindquist 1986). The posterior dorsal surface has a sucker-like organ that attaches to a quiescent nymph (Baker, 2012). Other features include a scapular setate sc_1 that is not longer than sc_2, a very large dorsal plate EF with two pairs of setae, a short dorsal idiosomal setae and four pairs of setae on the dorsum of the propodosoma (CABI 2018). The characteristics defined above are only visible with a compound microscope after the specimen has been mounted on a slide. Field identification of the broad mite, is less accurate, but can be accomplished with a hand lens or stereomicroscope before confirming through slide mounting and examination.

Life Stages and Field Identification

The life stages of *P. latus* include an egg, nymph, pharate nymph (also called quiescent) and adult female and male (Gerson 1992). Opaque white eggs are oval with flat bases glued to

the plant surfaces and have many distinctive raised white tubercles or spots (Fig. 1). The pale white nymph has three pairs of legs and is <100µm long. The white quiescent nymph is bound within an elongated elliptical shaped cuticle pointed on both ends (Fig. 1). The oval adult females have a dorsal almost hourglass shaped white stripe, four pairs of legs, and range from 100-200µm in length. The body color of adult females varies by age and plant host from yellow to pale green (Vacante 2016). On blackberry leaves (*Rubus L.* subgenus *Rubus*), newly emerged adult females appear pale to opaque white, and then develop into an amber color (Fig. 1). The adult male body is smaller than a female, has long spindly legs and can move quickly. The button-like claw on leg IV tibiotarsus is used to grab and manipulate a quiescent female and adhere it to a sucker-like organ on the posterior dorsal surface of the male. The quiescent nymph is held by the male for up to 24 hrs before emerging and mating (Baker, 2012) (Fig. 1).

Distribution

Polyphagotarsonemus latus is a cosmopolitan pest presumably distributed through shipping and commerce (Lindquist 1986). In 1890, specimens of the plant pest *P. latus* (originally named *Acarus translucens* Green) were first reported in Colombo, Ceylon (Sri Lanka) on tea plants. Later in 1904, *P. latus* (then named *Tarsonemus latus* (Banks)) was reported in the USA on figs and small mango plants at a port in New York (Lin et al. 2002). As of 2002, the genera *Polyphagotarsonemus* is found in all six zoogeographical regions of the world (Oriental, Palearctic, Australian, Neoarctic, Neotropical, Ethiopian), and is therefore an established world-wide pest (Lin et al. 2002).

Temperature Thresholds

While the broad mite has been reported worldwide, its ideal climatic conditions are tropical, subtropical and in greenhouse habitats where temperatures are warm and humid

(Lindquist 1986; Gerson 1992; Lin and Zhang 2002; Weintraub et al. 2003; Luypaert et al. 2014). Optimum temperatures for broad mite development range between 29-30°C. Lower and upper development thresholds (temperatures below or above which the mite cannot develop) are 10°C and 36°C, respectively (Luypaert et al. 2014, 2015). Continued exposure to 7°C reduced fecundity and causes mortality of eggs in 17 days and of larvae in 49 days (Luypaert et al. 2015). Exposure to 2°C for 26 days causes 100% mortality of adult females (Luypaert et al. 2015). These thresholds are of interest because the broad mites found on blackberries (the focus of this paper) are in fields with temperatures below and above these temperatures.

Damage and Hosts

Broad mites are thought to cause damage and deform developing plant tissues by injecting a toxin with saliva that allows imbibing of liquefied contents from epidermal cells (Lindquist 1986; Gerson 1992; Vacante 2016). It appears that this toxin remains in the plant's system for some time because damage can appear after removal of broad mites (Gerson 1992). Damage symptoms are initially on new growth, can be caused by very few mites ((Mechant et al. 2015) and vary among plant hosts. Symptoms include leaf curling (up or down), blistering, discoloration, wrinkling, rigidity, thickening, and darker spots; the stems can have shortened internodes and be necrotic or swelled; the buds can become distorted or drop; the fruit can become misshaped, cracked and scarred or with silver or bronze blemishes; and with continued damage, plants can die (Lindquist 1986; Gerson 1992; Vacante 2016).

Gerson (1992) listed 57 different known plant families damaged by broad mites (all were dicotyledons except Aracease and Orchidaceae which are monocotyledons). Since 1992, additional broad mite hosts have been reported. In 2007 and 2017 this list has expanded include blackberries (*Rubus sp*.) and strawberries, family Rosaceae (respectively) (Vincent et al. 2010;

Renkema 2017). These reports have caused an intensified interest in broad mite phenology and control on blackberries.

Blackberries

Worldwide, blackberries are an economically important crop consumed for excellent flavor and health benefits (Geisler 2015; NASS 2016; Hussain et al. 2017) Blackberries are biennial plants with some cultivars producing fruit on first year canes (named primocanes) and other cultivars producing fruit on two year old canes (named floricanes) (Strik and Thompson 2009). Initially broad mites were reported damaging primocane-fruiting cultivars (*Rubus* L. subgenus *Rubus*), but now have been reported damaging floricane-fruiting cultivars as well (Vincent et al. 2010; Rebek 2017).

Broad Mites on Blackberries

In 2007, the broad mite was first reported a pest of primocane blackberries (Vincent et al. 2010). Since 2007, *P. latus* damage to blackberries has been reported in the US states of Arkansas (Vincent et al. 2010), California, Illinois, Florida, Indiana, Maryland, New York, North Carolina, South Carolina, Tennessee, and Virginia (Johnson 2017 personal communication), Oklahoma (Rebek 2017), and Pennsylvania (Demchak and Johnson 2017), and California. China, South Africa and Mexico have also reported the presence of this pest on blackberries (Seagraves 2017 personal communication).

Broad mite damage was initially noted on developing terminals and laterals of blackberry canes. The symptoms include terminal leaf rigidity, discoloration, interveinal chlorosis, leaf cupping up or down and lateral bud blackening/dropping. Subsequent years of broad mite damage result in weakened floricanes with reduced or no yield and left unchecked, will result in plant death (Johnson et al. 2016; LeFors 2017; Rebek 2017).

Sampling

Sampling is an important aspect in determining the seasonal phenology of broad mites and there are many methods used to extract mites. The Tullgren funnel is very often used for leaves, soil and leaf litter (Macfadyen 1953; Faraji et al. 2004; Krantz and Walter 2009; George et al. 2017; Harris et al. 2017). Other mite sampling methods employ washing a sample such as leaves with water, ethanol, soap, or combined solutions with bleach and soap (Faraji et al. 2004; de Lillo 2010; Mechant et al. 2015). Floatation techniques to extract mites directly from substrates can use kerosene, water, olive oil and salt solutions (Hart and Fain 1987; Krantz and Walter 2009; Kuenen et al. 2009). Manual extractions from leaves can be performed with a mite brushing machine, through tapping or shaking and with a modified Winkler apparatus (Mechant et al. 2015; Semenina et al. 2015; Harris et al. 2017). The disadvantage with many of these methods is that debris is extracted along with the mites. This debris interferes with mite identification (especially microscopic mites such as the broad mite), thus making counting very time-consuming (Hart and Fain 1987; Faraji et al. 2004; Harris et al. 2017). Field samples from different parts of blackberry plants, leaf litter and soil need to be collected periodically throughout the year to conduct an accurate seasonal phenology study. Therefore, the Tullgren funnel is the ideal method as it can be used for all substrates. However, a method needs to be developed that separates broad mites, from sample debris to reduce sample processing time. If the field phenology of broad mites is known, management practices can then be tailored to control this pest.

Management Practices

Controlling broad mites in crops has been attempted with chemicals, exclusion, host resistance, and biocontrol agents such as predatory mites, fungus and bacteria (Martin et al.

2010; Luypaert et al. 2013; Duarte et al. 2015; Bajya and Ranjith 2016; de Saraiva et al. 2016; Lopez et al. 2017; Rodriguez-Cruz et al. 2017). Yet for broad mites in blackberry fields there are few miticides labeled for use in blackberry against broad mites and no other recommended tactics (Johnson et al. 2016).

Chemical

There are chemicals labeled or recommended for use against broad mites in many crops, but not caneberries. These chemicals include lime sulfur, abamectin, spiromesifen, azadirachtin, neem oil, and even N fertilizer combined with citric acid (Venzon et al. 2013; Breda et al. 2017; LeFors et al. 2017). In 2015, a comprehensive list of 37 chemicals evaluated against broad mites was compiled by Vacante (2016). Many listed had little to no activity against broad mites, caused a resurgence of broad mite populations or in the case of oxydemeton-methyl and imidacloprid, allowed resistance to build in broad mites (Vacante 2016). Sulfur, which has long been used against mites, has proven to be a viable option against adult and immature stages of broad mites, but it is not ideal as it is currently not labeled for broad mite control, reduces natural enemies of mites, and can cause phytotoxicity when temperatures exceed 32°C (Vacante 2016).

Current works are underway looking for novel and viable new miticides against broad mites. A new miticide, Cyenopyrafen 30% SC reduced broad mites by about 85% compared to controls on chilli (*Capsicum annuum* L.) which is promising (Bajya and Ranjith 2016). A study was conducted that determined the herbicide Glyphosate (Roundup Transorb®), increased oviposition rates of broad mites and caused a reduction in reproduction potential over time reproduction (de Saraiva et al. 2016). In 2009, Agri-Mek (abamectin) was labeled for use against broad mites in the citrus fruit group 10-10 and fruiting vegetables crop group 8. In 2017, a section 2(ee) recommendation (2016) and then a supplemental label were approved for use of

Agri-Mek against broad mites in the caneberry crop subgroup 13-07A. This compound remains the only labeled chemical for broad mites on blackberries as seen in the 2018 MP144 Insecticide Recommendations for Arkansas (Studebaker 2018).

Exclusion

Alternatives to chemical treatments such as exclusion, have promise but none have been proven effective in blackberries. Exclusion is used in many cropping systems to prevent pest injury. It is used in greenhouses to protect crops from *Bemisia tabaci* (Gennadius) white flies, in orchards to prevent *Cydia pomonella* (L.) (codling moth), *Conotrachelus nenuphar* (Herbst) (plum curculio) and many other crop pests (Berlinger et al. 2002; Chouinard et al. 2016). In West Africa, broad mite is a major pest of eggplant, *Solanum macrocarpon*. A dicofol-treated exclusion net set over the eggplant crop at night provided complete exclusion and control of broad mites and spider mites in West Africa (Martin et al. 2010). It is not known if this could be adapted for blackberries or other crops in the USA.

Host Resistance

Host resistance to broad mites is currently being investigated in several crops. Within *Rhododendron simsii* hybrids, a marker gene was found that increases expression in response to induced broad mite damage (Luypaert et al. 2017). There are also studies on aubergine, chilli, cotton, cucurbits, jute, and potatoes that are investigating broad mites resistance among genotypes, cultivars, or stomatal leaf densities (Vacante 2016). The most positive of these results were found through screenings of watermelon and chili cultivars. Certain varieties sustained less broad mite damage than others in the same plant species. This indicates a possible resistance that could be targeted (Tatagar et al. 2001; Kousik et al. 2007). The blackberry germplasm at the University of Arkansas Fruit Substation (Clarksville, AR) was examined in

2016, but none were found to be free of broad mites (Clark 2016, personal communication). Further research will determine if this can be applied to breeding programs for broad mite host resistance.

Predatory Mites

Biological control with agents such as predatory insects has become an important field of study due to recent regulations of pesticide residue on foods, the demand for less pesticide use, and the increase in cases of pesticide resistance (van Lenteren 2012; Calvo et al. 2015). Most reports of successful biological control of phytophagous mite species are with predatory mite species in the family Phytoseiidae (Hoy 2011). Additional families with predatory mite species are: Anystidae, Bdellidae, Cheyletidae, Cunaxidae, Erythraeidae, Labidostommatidae, Rhagidiidae, Stigmatidae, and Tydeidae (Krantz and Walter 2009).

Several Phytoseiid predatory mite species have been reported to control broad mites including *Amblyseius swirskii* (Athias-Henriot), *A. cucumeris* (Oudemans), *A. californicus* (McGregor), *A. herbicolus* (Chant), and *Neoseiulus barkeri* (Hughes) (Shipp and Wang 2003; Weintraub et al. 2003, 2007; van Maanen et al. 2010; Calvo et al. 2015; Duarte et al. 2015; Lopez et al. 2017; Rodriguez-Cruz et al. 2017). Vacante (2016) listed additional predatory mites feeding on broad mites including Bdeliid mites, *A. largoensis* Muma, *Euseius hibisci* (Chant), *E. nicholsi* (Ehara et Lee), *E. ovalis* (Evans), *E. stipulates* (Athias Henriot), *E. victoriensis* (Womeersley), *N. agrestis* (Karg), *N. longispinosus* (Evans), *Typhlodromalus peregrinus* (Muma), and *Typhlodromus athiasea* (Proath et Swirski). However, no predatory mite species have been identified or reported as feeding on broad mites in blackberry fields.

Entomopathogenic Fungi

There is evidence to suggest that entomopathogenic fungi have the potential to control broad mites. The fungus, *Beauveria bassiana* (Bals.-Criv), consistently produced 88% mortality of broad mites (Pena et al. 1996). Ashraf et al. (2011) found *B. bassiana* caused 87% mortality of broad mites 5 days after application. In 2016, 30 different isolates of the *Beauveria spp.* were tested on broad mites. One isolate (Unioeste 53) resulted in 70%, and 76.1% mortality in the laboratory and greenhouse (respectively) and 66% mortality in the field 12 days after application (Martins et al. 2016). Other fungi or isolates had varying results against broad mites: *B. bassiana*, *Hirsutella thompsonii* (Fischer), *Isaria fumosorosea* (Wize), *Metarhizium anisopliae* (Metschn.) and *Paecilomyces fumosoroseus* (Wize) (Smitha and Giraddi 2006; Vacante 2016). These or other fungal isolates may have potential to provide biological control of broad mites on blackberries or other crops.

Entomopathogenic Bacteria

Bacillus thuringiensis (*Bt*) is one of the most widely used entomopathogenic biological control agents and has potential to be used for broad mite control. *Bt* is widely known for its species specific insecticidal endotoxins produced within inclusion bodies (crystals) during the bacterial sporulation stage. Endotoxins from *Bt* strains are currently used to kill pest larvae in many insect orders (Lepidoptera, Coleoptera, Diptera, Hymenoptera, Thysanoptera, Mallophaga, Orthoptera), and other taxa such as intestinal flukes, protozoa, mites, and nematodes (Ibrahim 2010). *Bt* toxins cause mortality, reduced fecundity and reduced longevity of storage mites *Tyrophagus putrescentiae* (Schrank) (Ahmed et al. 2016) and dust mites *Dermatophagoides pteronyssinus* (Trouessart) (Gholamreza 2017). Two strains of *Bt* are patented in the USA against the two-spotted spider mite *Tetranychus urticae* (Koch) (Payne 1993, 1994). New strains

of *Bt* exist throughout the environment and have unknown properties, such as the strains of *Bt* isolated from varroa mite infected honey bee colonies, with unknown pathogenicity (Chandler et al. 2001). *Bt* has been assayed on broad mites, with demonstrated mortality, but this was with a *Bt* β-exotoxin, which is less desirable since it is not as specific as the crystalline endotoxins (Pena et al. 1996). Future research should focus on identification of crystal forming *Bt* strains, extraction and bioassays of these endotoxins against broad mite.

Objectives

Broad mites are an increasingly important pest of blackberries in the world. The goal of this study was to develop an integrated pest management (IPM) program for broad mites in blackberry by developing accurate sampling methods, identifying seasonal phenology of the broad mite in blackberry fields and testing the efficacy of chemical and biocontrol methods.

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Table 1: Glossary of taxonomic terminology used to describe *Polyphagotarsonemus latus* (Krantz and Walter 2009; Walter 2005)

Term	**Definition**
Gnathosoma	Anterior projection of the mite, contains the chelicerae, palpi, and pharynx
Chelicerae	Consist of a maximum of three segments: cheliceral base, digitus fixus, digitus mobilis (listed proximal to distally)
Palpi	Postoral appendices (often lateral to chelicerae), vary by species. Segments include: trochanter, femur, genu, tibia, tarsus (with or without claws, also known as apotele). Function in searching and handling food
Setae	Hair-like cuticular process emerging from a membranous socket on the outside of the mite
Pharynx	Acts as suction pump for food, shape can often be used for species identification
Idiosoma	Body of mite, divided transversely
Disjugal furrow	Line that divides the idiosoma in many species
Anterior propodosoma	Region of Idiosoma bearing the legs, behind the gnathsosoma
Posterior hysterosoma (or opithosoma)	Most posterior region of the idiosoma
Tergites	Below opithosoma (three main plates D, EF, H)
Stigmata	Openings of the tracheal system
Bothridium	Cup shaped base where a type of seta (trichobothrium) is inserted
Trichobothrium	Modified (usually) seta inserted into Bothridium
Apodemes	Sclerotized invagination of the cuticle, often found on ventral idiosoma delimiting the coxal plates
Presternal apodeme	Runs medially in center of mite on ventral side
Sejugal apodeme	Runs medially in center of mite on ventral side, is located medially at junction of leg II and III apodeme
Poststernal apodeme	Runs medially in center of mite on ventral side, located behind the sejugal apodeme
Sejugal suture	Imaginary line that divides the mite between legs II and III on the ventral side

Table 1 (Cont.)

Term	Definition
Leg Pairs	Listed anterior to posterior I, II, III, IV
Leg Segments	Basal to distal: coxa, trochanter, femur, genu, tibia, tarsus, pretarsus
Chaetotaxy	Use of setae present on surfaces of mites (including idiosoma, legs, and gnathosoma) to identify species
Holotrichy	Term for complete set of all possible setae

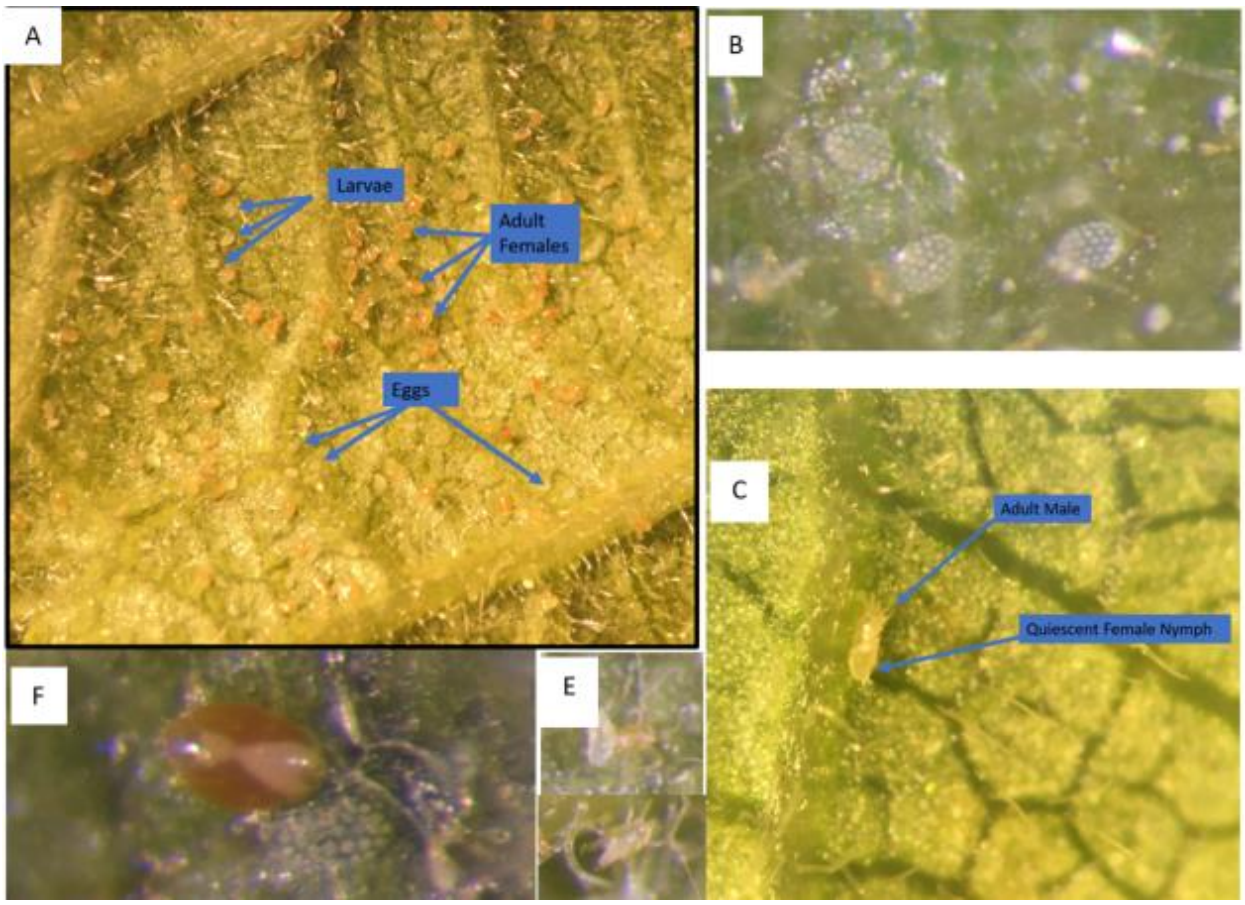

Figure 1: Broad mite, *Polyphagotarsonemus latus*, on underside of blackberry leaf (*Rubus L.* subgenus *Rubus)*: A) larvae, eggs and adult females, B) eggs, C) male holding quiescent female, D) male, E) male holding quiescent female and F) amber adult female showing hourglass marking.

Chapter 2: A Two Step Centrifugation Method with Water and Sucrose to Separate Mites from Raw Extracts of Tullgren Funnels

Little is known of the population dynamics of the pest *Polyphagotarsonemus latus* (Banks) within *Rubus* L. subgenus *Rubus* (Blackberry) fields. To understand *P. latus* populations, sampling needs to be conducted throughout the fields on multiple substrates such as leaves, canes, buds, leaf litter, and soil. The Tullgren funnel is an ideal sampling method for a population study as it can be used on all these sample types. However, during use of the Tullgren funnel it is common to have soil particulates in the collected samples. This makes quantification of *P. latus* difficult since it is microscopic and similar in size to the soil particulates. There are several methods that can be used to separate mites from soil based on either specific gravity or attraction of the cuticle to organic solutions. However, many of these methods require harsh chemicals, require multiple flotations or need considerable time. An efficient and simple method to extract *P. latus* from soil contaminates would improve the efficiency of population dynamic studies of this pest.

Nematodes are often extracted from soil with a sucrose and centrifugation method that utilizes the differences in specific gravity of the nematode and sucrose solution (called the Jenkins method). In this chapter the Jenkins methods was modified and found effective to remove mites in 12 different families or superfamilies, including Tarsonemidae, the family of *P. latus* from sediment laden samples.

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A Two Step Centrifugation Method with Water and Sucrose to Separate Mites from Raw Extracts of Tullgren Funnels

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Abstract

Tullgren funnels are commonly used when extracting mites from substrates for ecological and taxonomic studies. During this process, extracted mites are often contaminated with soil particles and other debris, making the processing of those organisms difficult, especially for the smaller mites, such as Tarsonemidae. The Water Sugar-Water (WSW) method, described here, is intended to facilitate the separation of the extracted mites from those fouling material. It consists of a modification of the Jenkins method used for the extraction of nematodes. Through a combination of centrifugation with two steps of floatation (first in water and then in 1.5 molar sucrose solution), this method separates mites from contaminants. In this study, the WSW method was tested in different conditions including three soil textures, two storage times (3 days or 6 months) and leaf litter. We also evaluated the possibility to use this method for separation of the broad mite, Polyphagotarsonemus latus (Banks) (Tarsonemidae), given its common occurrence in fields where the present work was conducted. Eight of nine treatments performed

consistently well with extraction efficiencies >95% (mites collected in relation to total mites present in the funnel extraction), with no significant differences among them. One treatment, using the addition of kaolin clay to the sample, retained some Phytoseiidae in the sediment pellets, reducing extraction efficiency to 88.3%. Mites of the taxa Acaridae, Cunaxidae, Eupodidae, Laelapidae, Phytoseiidae, Scutararidae, Tarsonemidae, Tydeidae, Rhodacaroidea and Orbatida were successfully removed from contaminated samples with this method. The frequency of these taxa was equally distributed between the water and sugar-water steps of the two-step floatation method. This method is fast, uses inexpensive and non-toxic compounds and has great potential for use to determine the presence of mite groups most commonly found in extractions of Tullgren funnels.

Key words: mites, nematodes, extraction, soil, litter, Tarsonemidae, Tullgren funnel

Introduction

Mites constitute one of the major groups of the edaphic mesofauna. These are greatly variable morphologically and biologically, exhibiting a wide diversity of feeding habits, including predation, fungivory, and phytophagy. Quantification of mites in various habitats is necessary for ecological studies, monitoring seasonal fluctuations and management practices with pesticides or biocontrol agents (Faraji et al. 2004).

Floatation and direct washing are often used to extract mites from sample substrates. Floatation techniques to extract mites directly from substrates such as soil utilize either the attraction of the cuticle to petroleum products or take advantage of the differences in specific gravity between mites and the solution (Kuenen et al. 2009). Petroleum products (kerosene, heptane, and carbon tetrachloride) have been used for floatation, but they can be harmful to researchers and require adequate ventilation; olive oil is a safe alternative but requires specialized equipment. Water and salt solutions take advantage of the differences in specific gravity of mites (1.2) when compared to the solution (Hart and Fain 1987; Krantz and Walter 2009a; Kuenen et al. 2009). In the NaCl method described by Hart and Fain (1987), a saturated NaCl (ionic) solution (with a 1.2 specific density) is used. Mites have a specific density of 1.2, but when soaked in ethanol their specific density is lowered, thus allowing their floatation with the salt solution (Hart and Fain 1987). Other methods employ washing samples such as leaves with water, ethanol (30% or 70%), soap, or combined solutions with bleach and soap (Faraji et al. 2004; de Lillo 2001; Rezende, J. et al. 2012; Mechant et al. 2015). During these washes, samples often become contaminated with soil particles and organic debris.

The Tullgren funnel can be used for leaves, soil and leaf litter (Macfadyen 1953; Krantz and Walter 2009a; Faraji et al. 2004; George et al. 2017; Harris et al. 2017). This is

advantageous in relation to other methods because it can be used in large scale agricultural and ecological sampling for mite ecology, population dynamics and pest management studies. However, during this process contaminates (such as soil particulates), often fall into the sample and interfere with quantification of mites. Improving the efficiency of extracting and separating mites from sample debris after processing with a Tullgren funnel is highly desirable, especially for mites of interest that are microscopic and similar in size to soil particles.

With a Tullgren funnel, the samples are placed on a screened platform in a funnel. A heat source (in this case a 15 Watt light bulb) is placed over the sample, which slowly dries it from the top down, promoting downward movement of mites, which are channeled along with small contaminating particles into a collecting jar containing a preserving liquid (often ethanol). Separating mites from these contaminants is a time-consuming process, especially for families of small mites, such as Tarsonemidae (Hart and Fain 1987; Krantz and Walter 2009a; Harris et al. 2017). Sucrose solutions are non-ionic, have a specific density of 1.2 at 1.5 M just as the saturated salt solution used by Hart and Fain (1987) and can have a specific density of up to 1.4 (around 5.5 M) when saturated. Therefore, a method using a sucrose solution could conceivably be used to remove mites from sediment in the extracted material obtained with the use of Tullgren funnels, in a similar manner as used by Albuquerque and Moraes (2008) to separate mites of stored products from their feeding substrates.

A centrifugal floatation method, referred to as the Jenkins method, is standard in many nematode diagnostic clinics. It extracts phytophagous nematodes from soil samples through a cleaning centrifugation in water followed by floatation with a sucrose solution in the initial phase of the process, to separate first the less dense contaminants (Jenkins 1964; Dropkin 1989).This method separates nematodes from heavier soil particles and yields a relatively soil-free sample

that can be easily examined microscopically (Jenkins 1964; Knapp-Lawitzke et al. 2014). Modifications of the Jenkins method have been applied to different taxa such as insect pests of seedling cotton (Micinski et al. 1995) and also modified to separate nematodes from different substrates such as roots and organic matter, with the addition of kaolin clay (Sarah and Boisseau 2008). Many phytophagous nematodes are soil dwelling roundworms 0.5–3.0 mm long, which is similar in size to many small mite taxa. Since sucrose can have a specific gravity range from 1.2 (1.5M) to 1.4 (5.5M), the Jenkins method could conceivably be modified to remove mites from sediment in samples.

The objective of the present work was to evaluate the efficiency of a modification of the Jenkins method, named the Water Sugar Water method (WSW), with different soil textures, samples stored for different time periods, the broad mite (Tarsonemidae) and leaf litter. The work was done within the context of a study on the ecology of the broad mite, *Polyphagotarsonemus latus* (Banks), an emerging pest of blackberry and strawberry plants in the USA (Vincent et al. 2010; LeFors et al. 2017; Renkema 2017) and associated mites within a blackberry planting.

Materials and methods

Water Sugar-Water Method

The WSW method is a two-step process using tap water and a sugar solution (1.5 M). The contents of the Tullgren funnel collecting jars (in this case 75% ethanol) are poured into a 500-mesh (24 µm) sieve and rinsed with water. Mites and accompanying debris are then backwashed from the sieve with tap water into a 50 mL centrifuge tube (final volume 45 mL). Each sample is stirred and centrifuged in a Thermo Scientific IEC Model K centrifuge for 5 minutes at 1500 rpm. The supernatant is decanted into a 500-mesh sieve and set aside, leaving

the sediment/mite pellet in the tube. A 1.5 M sugar solution is then added to the sediment pellet to a final volume of 45 mL, stirred, and centrifuged for 1 minute at 1500 rpm. The supernatant is poured through the same 500-mesh sieve that contains the contents of the previous water step. The nearly debris-free mites on the sieve are then rinsed with water and backwashed directly into a dish for counting or into a storage vial with either ethanol (70–100%) or water. With this method, eight samples can be assayed at one time (capacity of the centrifuge). When assaying all 8 samples at one time it takes about 45 minutes (which is about 6 minutes per sample) (Figure 1).

Prior to this method, counting contaminated samples took longer than an hour and required dilution into a minimum of 10 petri dishes. With the WSW method, samples are free of debris and take about 15 minutes or less to quantify. The total time required after the WSW floatation(s) to quantify the mites in each sample is determined by the number of specimens collected, and the expertise of the quantifier.

Both steps of the WSW method are necessary because in the first water step mites and the remaining debris are removed (backwashed) from the sieve with water. Varying amounts of water are required to backwash the sediment from the 500-mesh sieve. This could produce an inconsistent amount of water per tube and adding the sugar solution first would not have a consistent density. Therefore, the water is used first to remove mites and leave behind a pellet, and then the sugar solution is added for the remaining mites, keeping the extraction of mites consistent across all samples.

Given the higher contents of organic matter in leaf litter samples, in one of the following treatments (described below), the WSW method was modified slightly. The modification consisted of the addition of 1.0 cm^3 (0.26 g) of kaolin clay (Surround WP) to the centrifuge tube in the first step (centrifugation in water), as described by Sarah and Boisseau (2008) for removal

of organic material. The modification method is hereby referred to as the Kaolin Water Sugar-Water method (KWSW).

Soil Textures

In this study, soil samples representing three different soil types were used. All soil samples were analyzed by the University of Arkansas Soil Testing and Research Laboratory in Marianna, Arkansas. The soil textures used were: a) silt loam (SL): 20 to 50% sand, > 70% silt, < 30% clay, pH 6.3 and CEC 8.75 cmol/kg, from a plastic-covered high tunnel house (3.7 m high x 60 m long), with high *P. latus* density on primocane-fruiting blackberries at the University of Arkansas Southwest Research Extension Center (SWREC) in Hope, Arkansas (33°42'33.69"N 93°33'34.44"W); b) silt loam-silty clay loam (SLSCL): < 20% sand, 60 to 90% silt, < 40% clay, pH 6 and CEC 10.90 cmol/kg, from a broad mite-infested blackberry field near Providence, Arkansas (35°21'47.62"N 91°39'43.40"W); and c) sandy loam (SAL): > 50% sand, > 80% silt, < 20% clay, pH 5.8 and CEC 4.35 cmol/kg, from a lot of steam-pasteurized soil used in a greenhouse at the SWREC in Hope, Arkansas.

Soil

Soil samples (300 mL each) were collected from the top 2.5 cm of soil below blackberry plants at each location (mentioned above). SL samples were collected July 16 2016, August 25 2017, and September 8 2017. SAL samples were collected on August 25 2017, and SLSCL samples were collected on August 30 2017.

Leaf Litter

Samples of leaf litter were collected on September 16 2017 underneath heavily broad mite infested blackberry plants in the SL collection site. Leaves were packed tightly into a 300 mL beaker. The same volume of 300 mL was used for each Tullgren extraction.

Broad mites

P. latus, were obtained from a colony maintained on primocane-fruiting blackberries in a greenhouse at the SWREC maintained at 25°C with natural lighting. To collect mites, the infested leaves were washed with a 2% bleach (Great Value®) + 0.2% soap solution (Dawn®) as described for collecting eriophyid mites (Monfreda et al. 2010).

Experimental Design and Treatments

In total there were 9 treatments: 1) *P. latus* (SL), 2) *P. latus* (SLSCL), 3) *P. latus* (SAL), 4) Storage (3 days), 5) Storage (6 months), 6) Soil (SL), 7) Soil (SLSCL), 8) Leaf Litter (WSM), 9) Leaf Litter (KWSW). During these treatments, the WSW method efficiency was evaluated in three different soil types for *P. latus* mites (treatments 1–3), storage periods (treatments 4–5), naturally occurring mite taxa in two soil types (treatments 6–7) and leaf litter samples (treatments 8–9). Soil and *P. latus* treatments were repeated 3 times. Storage and Leaf litter treatments were repeated 5 times. In each replicate, 300 mL of soil or packed leaf litter were used for each Tullgren funnel, sediment contaminants fell into all the collected ethanol samples. Soil for treatments 1) *P. latus* (SL), 2) *P. latus* (SLSCL) and 3) *P. latus* (SAL) was first subjected to the Tullgren funnel. Then on June 16 2017, 10 mL of each soil type was placed in a 100 mL plastic storage bottle and topped with 75% ethanol to 50 mL. Subsequently, 121.2±33.4 broad mites were added to each storage bottle (mites were collected as previously described). Each bottle was stored at 7°C (the temperature of SWREC sample storage cold room) for three days before processing with the WSW method and quantification. The soil of treatments 4–7 and leaf litter of treatments 8 and 9, were subjected for 3 days to the Tullgren funnel, as described above and stored at 7°C for 3 days. Sediment contaminants fell into all the collected ethanol

samples. After the Tullgren funnel and three-day cold storage, treatments 4, and 6–8 were processed with the WSW method described above.

Treatment 5, was stored for six months at 7°C before treatment with the WSW method. Treatment 9 was processed with the KWSW method (as described above). For all treatments, the water step, sugar step and remaining sediment were backwashed individually with 75% EtOH into 100 mL plastic storage bottles and stored at 7°C, until the mites were counted. For mite quantification, storage bottles were poured into a Petri dish and examined with a Zeiss Stemi 2000-c stereomicroscope. For all treatments, the sediment pellets and each backwashed step (water and sugar) were counted individually.

Extracted mite taxa

After counting, the mites from treatments 4 and 6–9 were collected and mounted in Hoyer's medium on slides as described by Krantz and Walter (2009a). The slides were later examined under a compound microscope (Zeiss AXIO Scope A.1) and adult mites were identified to either superfamily or family as described by Krantz and Walter (2009b), except for non-Astigmata Oribatida, which were identified to suborder. The number of mites in each taxon was recorded individually from the water, sugar-water and sediment steps. Data is presented in Table 2. The mites from treatment 5 were accidently lost after counting and therefore not included in Table 2.

Statistical analyses

Data were analyzed with ANOVA, after log (x+1) transformation to best fit assumptions of linearity and homogeneity of variances. Treatments were compared with lsmeans and Tukey's studentized range test (HSD), with a significance level of $P<0.05$ (R Development Core Team 2017). The efficiency percent was determined from the number of mites recovered from each

sample by dividing the mean number of mites recovered from the water and sugar washes with the mean total number of mites counted from both washes and the sediment.

Results

The efficiency of the WSW method was consistent across all treatments with statistical differences from sediment compared to the floatation with water or sugar individually, and the combined water with sugar steps ($F_{3,132}$=141.4; P=<0.000). There were no statistical differences among broad mite treatments (1–3) ($F_{2,6}$=2.379; P=0.139), between storage length treatments (4–5) ($F_{1,8}$ =0.72; P= 0.402), or between soil treatments (6–7) ($F_{2,6}$=1.095; P=0.35). With the leaf litter treatments, the proportion of extracted mite was significantly lower when kaolin was added the WSW method (KWSW) ($F_{1,8}$ = 9.65; P= 0.004) (Table 1).

The extraction efficiency (as determined by total recovered mites) was 99% in all treatments except treatment 5 (Storage 6 months) and treatment 7 (KWSW) (Table 1). Extraction efficiency of treatment 5 was 95% and of treatment 7 was 88.3% (Table 1). After the WSW method all samples consisted of mites in ethanol without any sediment contamination.

At least ten mite families and multiple genera were extracted from samples with the WSW method (Table 2). Frequency of identified taxa were evenly distributed between the water and sugar water steps of the WSW method. Mites recovered from the pellet with the KWSW treatment were predominantly Phytoseiidae (1 Orbatid; 1 Tydeidae; 19 Phytoseiidae).

Discussion

The two-step floatation WSW method successfully extracted mites from samples of different soil textures, storage periods, and leaf litter. Two treatments had efficiencies below 99%; the KWSW (88.3%) and six month storage time (95%). The addition of kaolin allowed the removal of organic material in the first (water wash) phase, which was expected to improve

separation of mites in the second (sugar-water wash phase). However, the addition of kaolin lowered the efficiency by holding part of the mites (by far phytoseiids) in the sediment. Therefore, the WSW method works best with inorganic contaminates (soil particulates), without the addition of kaolin, and for samples stored less than 6 months. Depending on circumstances, the WSW method could be used on samples stored longer than 6 months but with a lessened efficiency.

Setae and other structures of the slide mounted extracted mites in this study had no or minor damage, allowing species identification. As it is possible that setae or other cuticle damage could occur during either the Tullgren funnel or floatation steps of the WSW method, this should be considered during detailed taxonomic studies.

During the WSW method other arthropod groups, such as Thysanoptera, Formicidae and Coleoptera, were found in small amounts in the cleaned samples. In this study, they were easily removed or ignored when mites were counted. In areas with high numbers of these taxa, this could interfere with quantification, and the use of a larger mesh sieve above the 500 mesh sieve could be helpful, as used by de Lillo (2010).

Mites were extracted during both steps of the water and sugar floatation steps. This demonstrates that water alone does not remove all mites from these substrates effectively. With water or kerosene decantation, as described in Krantz and Walter (2009a), the samples must be repeatedly decanted until mites stop floating to the surface. For microscopic mites, such as those in Tarsonemidae, this could be difficult to determine. Therefore, the WSW method which has two decanting steps, is an improvement. The WSW method does not use harsh chemicals and unlike the saturated salt solution, the specific density of the sucrose step can be increased easily if needed in future studies. This method is expected to reduce the time required to estimate

densities of mites in samples from different habitats and substrates. Thus, it has great potential for future use with mite ecology, population dynamics and pest management studies.

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Table 1: Mean total numbers of mites (± SE) extracted during each step of the two-step floatation water, sugar-water method (WSWM): first rinsed with water (W), second rinse with sugar-water (SW), combined rinses (W+SW), sediment and percentages of mites recovered. Soil textures were: SL= silty loam; SLSCL = silty loam-silty clay loam; and SAL = sandy loam. Means in a column followed by same letter are not significantly different (P > 0.05, Tukey's).

	Steps of two-step floatation water, sugar-water method				**%**	***P***
# Treatment	**Water (W)**	**Sugar-Water (SW)**	**W+SW**	**Sediment**	**Recovered**	**value**
1 *P. latus* (SL)	54.7±3.5	50.3±8.8	105.0±9.5a	0	99.9	0.35
2 *P. latus* (SLSCL)	35.0±19.0	100.0±17.5	135.0±36.5a	0	99.9	
3 *P. latus* (SAL)	37.0±10.0	55.0±21.4	92.0±31.3a	0	99.9	
4 Storage (3 Days)	89.2±20.20	124.8±37.54	214.0±55.4a	0	99.9	0.402
5 Storage (6 Months)	40.6±12.68	107.4±14.36	148.0±22.31a	7.2±2.15	95.0	
6 Soil (SL)	52.0±9.9	28.3±2.8	80±8.0a	0	99.9	0.139
7 Soil (SLSCL)	71.3±26.6	38.0±8.0	109.3±34.0a	1.0±1.0	99.0	
8 Leaf Litter (WSW)	61.0±25.3	154.4±41.6	215.4±39.6a	0	99.9	0.004
9 Leaf Litter (KWSW)	15.6±4.1	8.6±2.0	24.2±5.6b	3.2±1.1	88.3	

Table 2: Numbers in parentheses indicate the total number of adult mites extracted from all samples in different mite superfamilies (Rhodacaroidea) or families. Soil and leaf litter samples were processed in Tullgren funnel and mites were extracted, separated from sample debris and counted after each floatation step of the water, sugar-water method (WSWM). Samples were taken from two blackberry fields in Arkansas. This table does not include immature mites that could not be positively identified.

	Individual Floatation Steps of WSWM		
High mite group	**Water**	**Sugar-Water**	**Sediment**
Astigmatina	Acaridae (8)	Acaridae (3)	
Mesostigmata	Laelapidae (1) Phytoseiidae (34) Rhodacaroidea (16)	Phytoseiidae (39) Rhodacaroidea (9)	Phytoseiidae (19)
Orbatida	(20)	(37)	(1)
Prostigmata	Cunaxidae (1) Eupodidae (3) Scutacaridae (2) Tarsonemidae (12)	Cunaxidae (3) Eupodidae (3) Scutacaridae (1) Tarsonemidae (21) Tydeidae (3)	Tydeidae (1)

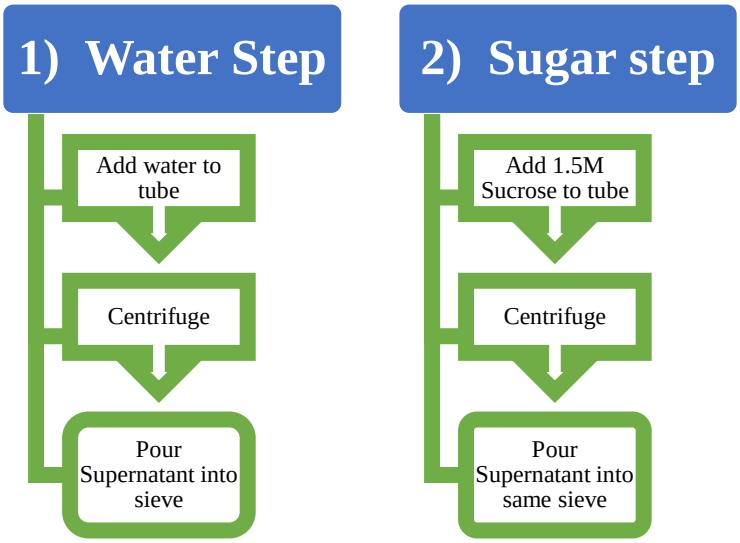

Figure 1: Two step mite floatation. A soil or leaf litter sample processed in a Tullgren funnel into 75% ethanol is poured through a 500 mesh (24µm) sieve. Retained sediment with mites is backwashed with 1) water, centrifuged, 2) supernatant poured through sieve, backwashed with sugar-water, centrifuged and this supernatant with extracted mites is poured into a counting dish.

Chapter 3: Seasonal Changes in Location of *Polyphagotarsonemus latus* (Banks) in Primocane-Fruiting Blackberry Fields in Arkansas

Polyphagotarsonemus latus (Banks) has been reported damaging blackberries in 10 US states, China, South Africa, and Mexico. Many of these US states have temperatures below the broad mite lower developmental threshold (10°C), so the continued yearly presence of broad mites in these areas is currently unexplained. Determining the broad mite seasonal densities and location within the blackberry fields is an important information for developing management systems.

In this chapter, two blackberry fields with known persistent broad mite densities were used. In the first field, leaves, canes, leaf litter and soil samples were taken in two consecutive years during the cold periods of the season. In the second field, leaves, leaf litter and soil samples were taken bimonthly for a nine month period that included the winter season. In both fields, broad mites were found predominantly in the leaf litter during the cold seasons.

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Seasonal Changes in Location of *Polyphagotarsonemus latus* (Banks) in Primocane-Fruiting Blackberry Fields in Arkansas

Abstract:

Polyphagotarsonemus latus (Banks), was discovered damaging primocane-fruiting blackberries (*Rubus L. subgenus Rubus*) in Arkansas in 2007. Since then, it has been reported on blackberries in 10 USA states, China, South Africa, and Mexico. Many US states have temperatures below the broad mite lower developmental threshold (10°C). Very little is understood about the seasonal phenology of these mites in blackberry fields or how they are surviving these cold temperatures. On February 18 2016 and January 26 2017, soil, leaf litter, and whole plants (above the crown) were removed from an Arkansas blackberry field. These plants were divided by plant canopy sections (top, top lateral, middle, middle lateral, base, base lateral). Biweekly from November 2016 to July 2017, leaves, leaf litter, and soil samples were collected from a second blackberry field in Arkansas. During January and February, both sites had significantly more broad mites ($P<0.001$) in the leaf litter than in soil or elsewhere on blackberry plant samples; there were no statistical differences of pest density among plant canopy sections ($P=0.52$) at this same time. Broad mites were found primarily in leaf litter during January and February. After this season, their densities were very low in all plots from March 1 to May 11 2017. On May 2017, just prior to widespread field dispersion (June 12 2017), broad mites were found highly aggregated within one plot. These data will help in designing a broad mite management program.

Introduction:

Worldwide, blackberries are an economically important crop consumed due to their favorable flavor and health benefits (Strik and Thompson 2009). In 2007, the broad mite, *Polyphagotarsonemus latus* (Banks), was discovered damaging primocane-fruiting blackberries (*Rubus L. subgenus Rubus*) in Arkansas (Vincent et al. 2010). Since then, broad mites have emerged as a significant pest of blackberries, causing yield loss by damaging cane terminals (the site of berry formation). In the USA, there are few control methods, and only Agri-Mek® (abamectin) has been registered to control broad mites on caneberries (*Rubus sp.*) (LeFors et al. 2017). Currently, blackberry broad mite damage has been reported in the USA in Arkansas, California, Illinois, Indiana, Maryland, North Carolina, South Carolina, Tennessee, Virginia (Johnson 2017 personal communication), Oklahoma (Rebek 2017), Pennsylvania (Demchak and Johnson 2017), and on strawberries in Florida (Renkema 2017). Additionally, growers in China, South Africa and Mexico have reported the presence of this pest on blackberries (Seagraves 2017 personal communication).

Blackberries are perennial plants that (depending on the cultivar) produce fruit on canes that are either floricane or primocane-fruiting. The canes of floricane-fruiting cultivars grow for two years. The first year is vegetative and during the second year, the cane bears fruit. Primocane-fruiting canes grow and produce fruit in the same year (Strik and Thompson 2009). Initially broad mites were reported damaging primocane-fruiting cultivars but now have also been reported damaging floricane-fruiting cultivars (Vincent et al. 2010; Rebek 2017). Symptoms of broad mite damage can vary among plant hosts. Plant hosts such as bean, citrus, cotton, papaya, peppers, strawberries, tea, and many ornamentals exhibit bronzed, scorched, blistered and shriveled leaves, shortened stems and distorted internodes or fruit (Gerson 1992;

Renkema 2017). Blackberries exhibit damage on the cane terminals. Symptoms are terminal leaf rigidity, discoloration, interveinal chlorosis, leaf cupping up or down, lateral bud blackening/dropping, weakened floricanes, lessening of yield, and can manifest as a complete loss of fruit on the canes (Johnson et al. 2016; LeFors 2017; Rebek 2017) (Fig. 3).

Currently broad mite management options on blackberries are limited. In 2016, Agri-Mek® SC was labeled for this broad mite and is the only recommended miticide. However, it can only be used 2 times during a season. Sulfur is also used although not labeled specifically for broad mites. Other options such as M-Pede, Trilogy, JMS Stylet Oil and Quillaja have been tested with promising results. To date, these formulations have not been field tested (LeFors et al. 2017). Biological control agents such as the predatory mites *Amblyseius swirskii* Athias-Henriot, *Neoseiulus* (=*Amblyseius*) *cucumeris* (Oudemans), and *N. californicus* (McGregor) have been found to control *P. latus* on economically important crops, but to date, their effectiveness on blackberry cultivars has not been reported (Shipp and Wang 2003; Weintraub et al. 2003, 2007; van Maanen et al. 2010; Calvo et al. 2015).

The broad mite has been found in almost every zoological region; reporting to thrive where temperatures are warm and humid such as tropical, subtropical and greenhouse habitats (Gerson 1992; Lin and Zhang 2002; Lindquist 1986; Luypaert et al. 2014; Weintraub et al. 2003). Broad mite optimum developmental temperature is 30°C. The lower and upper development thresholds are 10°C and 36°C, respectively (Luypaert et al. 2014, 2015). Cold exposure below 7°C reduces fecundity and continued exposure for 17 days or 49 days, respectively, caused mortality of eggs and larvae (Luypaert et al. 2015). Adult females had 100% mortality at 2°C after 26 days (Luypaert et al. 2015). Therefore, it is surprising that *P. latus* overwinters in Arkansas blackberries since Arkansas winter temperatures often drop below

to 2°C (Luypaert et al. 2015; NOAA 2017a; NOAA 2017b). These temperatures are of interest since many states reporting broad mites on blackberries experience potentially lethal low winter temperatures.

The objective of this study was to identify where *P. latus* overwinter and describe the subsequent seasonal phenology and dispersal of *P. latus* in Arkansas blackberry fields.

Materials and Methods

In this study a winter sampling of canes, leaves, leaf litter and soil were harvested from a location (Site A), to determine within plant canopy distribution and within field distribution of broad mites at low temperatures. A survey of broad mite populations in leaves, leaf litter, and soil was conducted for 9 months at a different location (Site B), to determine seasonal phenology of broad mites during the winter. Both locations were in Arkansas and were plantings of primocane-fruiting blackberries with prior broad mite presence and yearly significant plant damage. In both locations voucher slides were made of selected samples and identified to species (*P. latus*) as described in Krantz 2009.

Sample sites

Site A (Winter Sampling Location), was a 1 ha commercial, Prime-Ark® 45 blackberry planting near Judsonia AR, (35°22'28.43"N 91°39'47.75"W) with silt loam-silty clay loam soil. This planting had 15 rows, spaced 3 meters (m) apart that varied from 90 to 130 m long. Each row of planting was on a slightly raised bed with plants spaced 0.6 m apart. Trellis posts were spaced 7.5 m apart in the row and served to delineate each sample plot (12 plots per row). From 2008 to 2012, the historical maximum and minimum temperatures in Judsonia were 9.5°C and -1.6°C in January and 12.5°C and 0.0°C in February (Wilson et al. 2007).

Site B (Seasonal Study Location), was at the Southwest Research and Extension Center (SWREC) in Hope, AR (33°42'34.38"N 93°33'35.25"W). This site had Prime-Ark® Freedom blackberries planted in sandy loam inside a high tunnel (60 m L x 9 m W x 3.7 m H) covered with 6 mm thick clear greenhouse film. Inside the tunnel there were three, 50 m rows spaced 2 m apart with 1 m wide landscape fiber mulch in walkways and 0.5 m wide-open soil strip on either side of planted row. Each trellised row was divided into three, 15 m plots (9 replicates). Plants were spaced 0.5 m apart. Ambient daily maximum and minimum temperatures were recorded by a Watch Dog Weather Station located at the SWREC (Fig. 1). During the period of December 2016 to February 2017, the average low temperature ranged from -0.5 to -2.0°C at this site (Fig. 1).

Cane Sectioning

To determine within canopy distribution of *P. latus*, all collected canes were divided into 6 different plant sections, then leaves were removed. Canes and leaves were bagged and labeled individually by section. Sections were as follows: top cane (main cane terminal 61 cm); top lateral (laterals removed 30 cm from the top cane); middle cane; middle lateral (laterals removed from the middle cane); base cane (lower 61 cm from the crown); base lateral (laterals removed from the base canes) (Fig. 4).

Winter Sampling (Site A)

On February 18, 2016, a preliminary survey was carried out. Leaves, leaf litter, soil, canes and buds were randomly collected from four rows. All collections were pooled by sample type (leaves, leaf litter, soil, buds and canes) after sectioning as described above. Buds were excised from cane samples. Each sample was placed on a Tullgren funnel equipped with a 15W light as originally described by Macfadyen (1953) and detailed in Krantz and Walter (2009). A

75% ethanol solution was used in the collection jar (Macfadyen 1953) and mite numbers were recorded with aid of a Zeiss Stemi 2000-c stereomicroscope (Carl Zeiss Microscopy, LLC, Peabody, MA).

On January 26, 2017, an intensive survey was conducted through sampling of blackberry canes, leaves, leaves litter, and soil from four random rows. At this time, canes were quite varied in length and size across all rows. Therefore, canes were sampled from every other plant along the entire row to ensure there was a sufficient sample size to divide into plant sections. Leaf litter and soil samples were taken from three random plots within each row (12 plots sampled total). Whole canes were removed by pruning directly above the crown (about 35 canes per row). Leaf litter was harvested directly beneath the canopy of blackberry plants (enough to fill a 3.8L plastic bag), soil was collected from the top 1.25 cm of the resulting exposed surface (enough to fill ½ of the 3.8L plastic bag). All mites for this test were extracted via a Tullgren funnel as previously described. Because prolonged refrigeration lowers the extraction efficiency of mites with a Tullgren funnel (Lakly and Crossley 2000), all samples were processed within 1 week after collection. To accomplish this timeline, and due to the limited space inside each funnel, mites were extracted from half of each leaf, leaf litter and cane samples; and 300cc of soil was used per collected soil sample. Dry weights were taken of all processed samples and mites were reported as mites/gram of plant material and per cc of soil.

Samples from the Tullgren funnel had soil particulates which made quantification of small mites such as *P. latus* difficult. Therefore, all samples were further processed with the two-step floatation water sugar-water method to separate mites from leaf litter and soil sample debris (LeFors 2018). All samples were kept in 75% ethanol at 7°C (the temperature of the sample storage cooler at the SWREC) until mites could be counted as previously described.

9 Month Survey

Site B: Leaves, leaf litter and soil were sampled biweekly for nine months (November 2016-July 2017) (Fig. 1, and Table 4). Every two weeks, in all nine plots, ten of the most distal fully opened leaflets, leaf litter and soil from under the blackberry plants were collected. Leaf litter and soil were collected into a 300cc beaker. Soil samples were processed in a Tullgren funnel. Leaf litter and leaflets were washed with a 2% bleach/0.2% soap solution (de Lillo 2010). All collected samples were cleaned with the water sugar-water method (LeFors 2018) and stored in 75% ethanol at 7°C until they were counted as described previously. Ten samples from the study, were randomly selected for preservation on slides in Hoyer's media (Krantz and Walter 2009).

Statistics

Statistical analysis was completed with R (R Development Core Team 2017) using ANOVA, and mean separation by LSMEANS with Tukey's t-test ($P > 0.05$) (R Development Core Team 2017). Data transformed with log+1 best fit all assumptions of linearity and was used for analysis. Site A data were analyzed as numbers of broad mites per g of dry plant material. Site B was analyzed by total number of mites per sample (leaves, leaf litter, soil).

Results

Site A: On 18 February 2016, the preliminary survey found the highest numbers of broad mites per gram of sample in blackberry leaf litter compared to lower numbers in blackberry buds, blackberry canes, blackberry leaves, and soil.

Another collection on January 26, 2017, found that a gram of a leaf litter had significantly ($F_{3,28}=40.64$; $P<0.001$) more broad mites (7.9 broad mites) than a gram of soil (2.1 broad mites), blackberry leaves (1.1 broad mites) and blackberry canes (0.5 broad mites). The

six plant sections had similar counts that were all less than 1.7 broad mites per gram of sample (Table 4) ($F_{11,84}$=2.1; *P*=0.052) (Fig. 4, Table 2). When samples from all plant sections were combined, and analyzed by leaves and canes, blackberry leaves alone had significantly ($F_{1,46}$=1.18; df=1; P=0.01) more broad mites than a gram of blackberry canes without leaves (Table 2).

Site B: During the biweekly sampling period, there was a significant sample type by date interaction ($F_{16,34}$=11.17; *P*<0.001). Season mean total numbers of broad mites were significantly ($F_{2,150}$= 104.7; *P*<0.001) higher in leaf litter (127.1 mites) than in blackberry leaves (51.4 mites) and soil (38.0 mites) (Table 3). These differences were seasonal and occurred mostly when the minimum daily temperatures were below 10.0°C from 20 December 2016 to 30 March 2017, when leaf litter had more mites than other sample types (Fig. 1). Most of the extracted broad mites were adult females, with a few larvae and eggs seen (but not counted).

There were seasonal changes in the biweekly average numbers of broad mites extracted from samples of blackberry leaves, leaf litter and soil collected from a tunnel house. Starting on 1 December 2016, there were 300 broad mites per blackberry leaf plot sample. Thereafter, counts dropped to about 50 broad mites on 20 December and dropped to zero broad mites on 23 February 2017. Broad mite counts from leaf litter plot samples remained above 250 broad mites from 20 December 2016 to 8 February 2017, thereafter it dropped to 50 on 23 February and reached a low of 10 broad mites on 30 March. On 8 February, leaf litter samples averaged 337 broad mites per plot across most blackberry plots with less than 28 broad mites per plot from blackberry leaves or soil samples (Fig. 2A, Table 4). On 13 March, leaf litter samples dropped to an average of 40 broad mites per plot compared to < 8 mites per plot from leaves or soil samples (Fig. 2B, Table 4). On 30 May, very few broad mites were in leaf litter or soil (< 12

mites) but blackberry leaf samples had increased to an average of 65 broad mites per plot. This average was skewed because eight plots had < 20 broad mites total, per plot, compared to the other plot that was a hot spot exceeding 500 broad mites per plot (Fig. 2C, Table 4). By 12 June, the distribution of broad mites was more uniform across the field and the average increased slightly to 100 broad mites per plot with foliar damage throughout the planting (Fig. 2D, Table 4). In contrast, soil samples experienced an increase to 193 broad mites per plot and leaf litter increased to 28 broad mites (Table 4). On 11 July, broad mite counts increased in leaf litter to 273 broad mites, and dropped to 84 and 48 broad mites, respectively, in soil and blackberry leaf samples (Fig. 1, Table 4).

Discussion:

In site A, broad mites were at the highest density in leaf litter on the sample date, and they were not aggregated within any sections of the plant canopy. This could suggest broad mites are surviving the colder months in leaf litter. This pattern was also found in the seasonal study, Site B (Fig. 1). It was not until 11 May 2017 that broad mites were detected on blackberry leaves. The subsequent sample dates had foliar damage appeared two weeks later as a hot spot in the planting then two weeks later higher broad mite counts and damage occurred uniformly across the blackberry planting.

Whether they are experiencing physiological changes during this time remains unknown. It is possible they are dormant, or less likely, in an induced form of adult diapause, even though Tarsonemidae mites are not known to experience diapause (Lindquist 1986). It could also be theorized that changing climatic trends are allowing broad mites to survive in new areas in the ways discussed above. The total broad mites per plot (leaves, leaf litter, soil) dropped from a mean of 388 on February 8 to the mean of 5.8 broad mites per plot on March 13. After this drop,

mean numbers remained low until June. A possible explanation of this trend could be a form of reduced fecundity from low temperatures during December and January.

The exact method of broad mite dispersal in blackberry fields is unknown at this point. However, it should be noted that broad mites were found in higher numbers in soil samples just prior to their widespread dispersal within the field. Average broad mite counts per soil sample (192 mites) on 12 June exceeded counts per blackberry leaf sample (66 mites). Dispersal coincided with broad mite numbers becoming uniformly distributed across the blackberry planting by 12 June (Fig. 1, Fig. 2D). The fact that on June 12 broad mites were found one time in the soil samples, could indicate broad mites are dispersing via crawling over the ground or aerially (falling to ground after wind dispersal, then finding the nearest available plant). However, broad mite aerial dispersion was not found in a preliminary study as described by Palevsky et al (2001).

In Palevsky et al. (2001), broad mites were found dispersing via phoresy predominantly on whiteflies and there was one report of broad mite phoresy on *Frankliniella occidentalis* (Thrips). It should be noted that whiteflies were not seen in any blackberry plantings used during this study, but populations of Eastern flower thrips, *Frankliniella tritici* (Fitch), were seen on blackberry leaves and fruits during May and June in these plantings (Johnson and Lewis 2003). Currently, how they are dispersing and the trigger for dispersal remains unknown.

During the initial survey in 2016, buds were excised from collected canes and placed on a Tullgren funnel, and canes were placed on a separate funnel. Broad mites were found in bud samples and none were found on the canes. In the future, buds should be investigated as another potential habitat/shelter for broad mites in colder climates.

P. latus densities were high in leaf litter from June 26 through July 27. During this time the ambient low temperatures were between 15.5-21°C after a sustained low temperature of 21°C (Fig. 1). This field was located in a tunnel house where temperatures were higher than ambient temperatures due to the plastic sheeting cover. While the exact temperature in the high tunnel was unknown, it is likely, the temperatures were above the broad mite upper developmental threshold (as previously described). Therefore, it could be theorized that the mites moved to leaf litter yet again to avoid the high temperatures, or that they simply fell as the temperatures became too high. The temperature in the tunnel house did not stay above the broad mite upper developmental threshold which allowed broad mites to remain and feed on leaves.

These findings have applications to future pest management of the broad mite. The fact that overwintering broad mites were found primarily on leaf litter in Arkansas suggests applying miticide foliage in late fall to lessen density of overwintering broad mites. In late winter or early spring, leaf litter could also be removed before broad mites begin to reproduce and disperse. In late spring, finding broad mites on soil or horizontal sticky cards just prior to widespread increase in pest density could lead to a soil miticide application. In addition, the mechanism of survival during the winter and possible physiological triggers that lead to dispersal could be future focuses for research. All mentioned future studies could lead to new and better methods for managing *P. latus* and preventing damage in blackberry fields.

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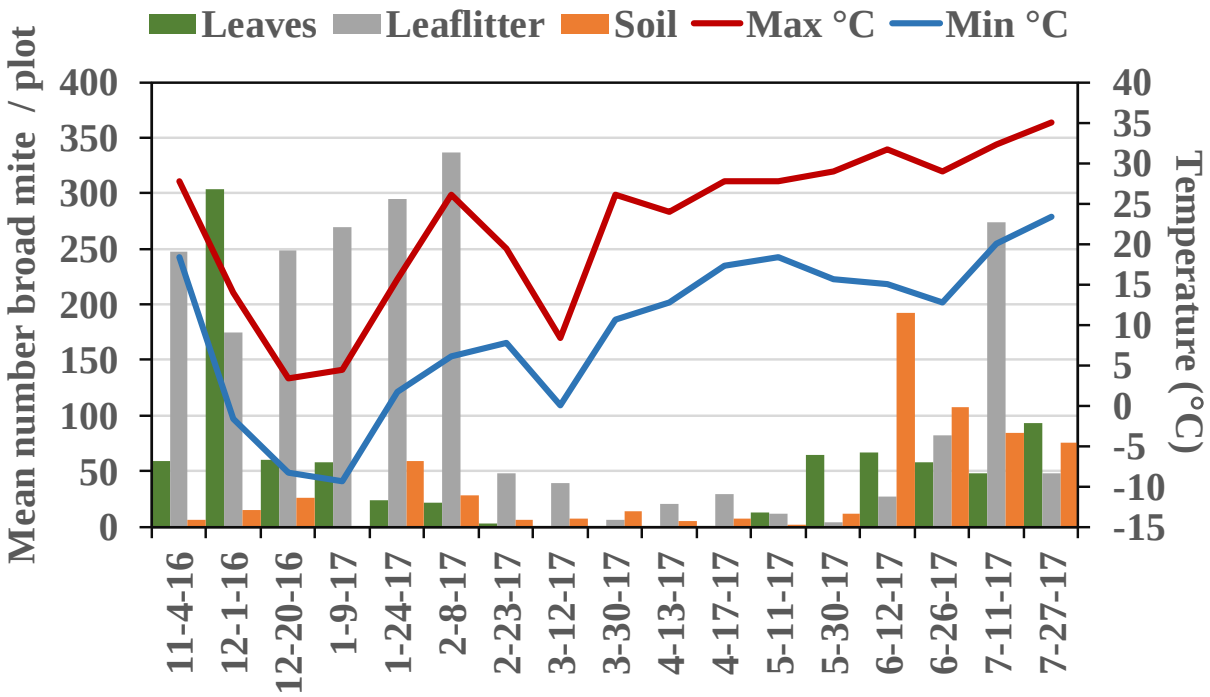

Figure 1: Weekly mean numbers (N = 4) of broad mites per plot from samples of blackberry leaves on cane and leaf litter or soil under blackberry plants in the row versus ambient daily minimum and maximum air temperatures recorded on sample dates at the Southwest Research and Extension Center in Hope, AR.

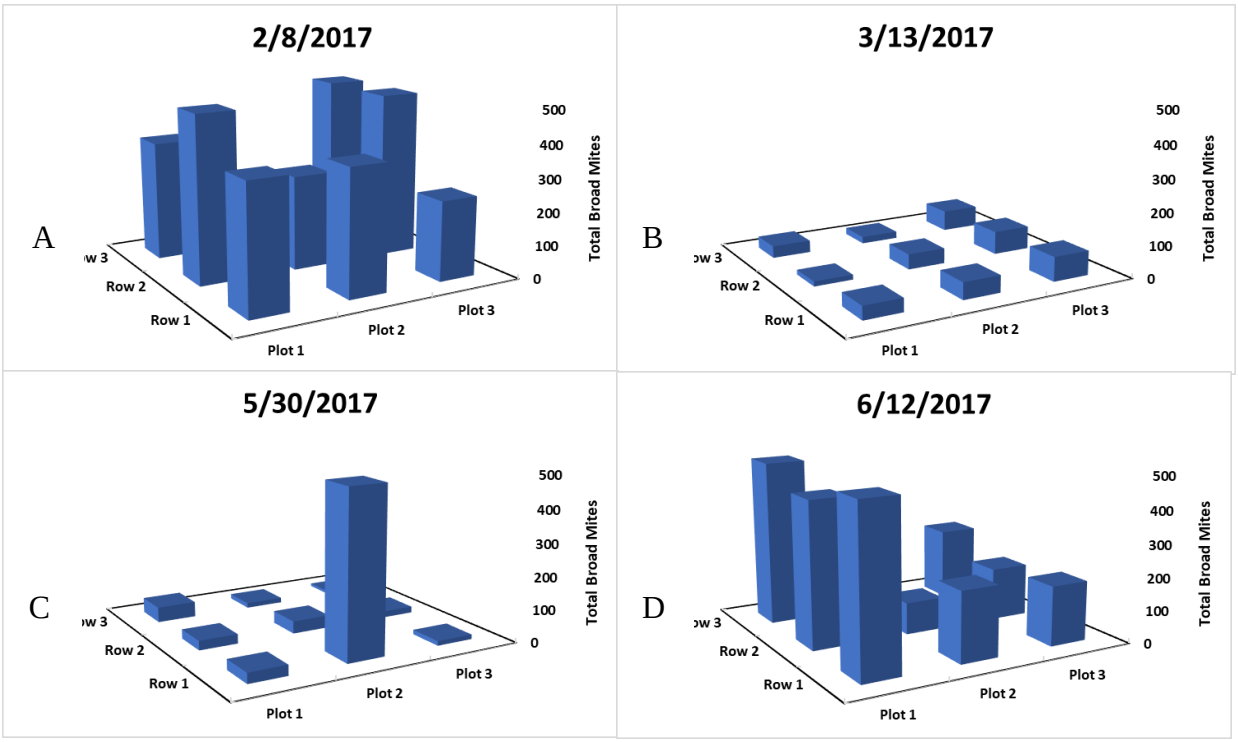

Figure 2. Four sample dates noting seasonal differences in plot to plot total numbers of broad mites per plot (including leaves, leaf litter and soil samples) and dispersion among three 15 m plots in each of three rows of Prime-Ark® Freedom® plants in a high tunnel house located at the Southwest Research and Extension Center in Hope, AR (2017).

Figure 3: Left and middle picture show broad mite damage on floricane-fruiting blackberries at the Cimarron Valley Research Station in Perkins, OK. Right Picture shows primocane-fruiting broad mite damage Photos: Becky Carroll, OSU; Jessica LeFors, Univeristy of Arkansas

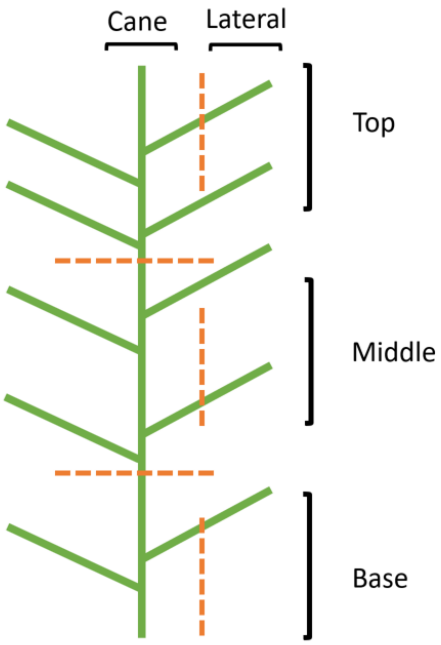

Figure 4: 6 Sections of Canes used for within plant canopy distribution of broad mites. Plant sections are Top, Top Lateral, Middle, Middle Lateral, Base, Base Lateral. Top is the upper 61 cm of cane, base is lower 61 cm of cane, middle is section between. Lateral is 30 cm from central cane.

Table 1. Mean numbers (±SE) of broad mites per gram of blackberry leaves, canes, leaf litter and soil collected from row (N = 4) of Prime-Ark® 45 primocane blackberries near Judsonia, AR (January 26, 2017).

Sample	Mean number *P. latus* (±SE) per gram of sample
Leaf litter	7.9±1.45a
Soil	2.1±0.70b
Blackberry leaves	1.1±0.23bc
Blackberry canes	0.5±0.09c

Mean values followed by the same letter are not significantly different (Tukey's t-test, $P > 0.05$).

Table 2: Mean numbers of broad mites (±SE) per gm sample of canes or leaves removed from the base, middle, or top of the main cane (Cane) or from a lateral cane (Lat) from Prime-Ark 45® primocane blackberry row in Judsonia, AR (January 26, 2017).

Sample location	Mean number broad mites per gm sample
Cane Base	0.3±0.05
Cane Base Lat	0.3±0.06
Cane Middle	0.5±0.10
Cane Middle Lat	0.3±0.14
Cane Top	0.5±0.19
Cane Top Lat	0.8±0.46
Leaves Base	1.3±0.35
Leaves Base Lat	0.4±0.08
Leaves Middle	2.2±1.07
Leaves Middle Lat	0.5±0.19
Leaves Top	1.6±0.65
Leaves Top Lat	0.8±0.11
All Canes	0.5±0.09b
All Leaves	1.1±0.23a

Mean values followed by the same letter are not significantly different (P > 0.05, Tukey's t-test).

Table 3. Season mean numbers (±SE) of broad mites per sample of blackberry leaves, leaf litter and soil collected from row of Prime-Ark 45® primocane blackberries near Hope, AR (November 11 2016 to July 27 2017). Mean values in column with same letter are not significantly different (Tukey's t-test, P > 0.05).

Sample	Season mean number of broad mites (±SE) per sample
Leaf litter	127.1±13.4a
Blackberry leaves	51.4±9.9b
Soil	38.0±5.7b

Table 4. Mean numbers (±SE) of broad mites from each sample date per whole sample (per plot) of blackberry leaves, leaf litter or soil collected from Prime-Ark® Freedom primocane-fruiting blackberries in high tunnel at the Southwest Research and Extension Center in Hope, AR. Mean values in same row with same letter are not significantly different on that date (Tukey's t-test, P > 0.05). Letters are assigned based on lsmeans.

Date	Leaf Litter	Leaves	Soil	F	P Value
11/14/2016	247.3±84b	59.0±31ab	6.6±3a	$F_{2,18}$=7.551	P=0.004
12/1/2016	174.8±58b	304.2±93b	14.8±6a	$F_{2,24}$=17	P=<0.001
12/20/2016	248.1±59c	60.0±15b	26.0±9a	$F_{2,24}$=20.68	P=<0.001
1/9/2017	269.8±74c	57.6±17b	0.0±0a	$F_{2,24}$=137.4	P=<0.001
1/24/2017	294.8±54c	24.0±5a	58.9±14b	$F_{2,24}$=31.76	P=<0.001
2/8/2017	336.7±55b	22.0±9a	27.7±17a	$F_{2,24}$=31.11	P=<0.001
2/23/2017	48.4±21b	2.9±2a	5.8±1a	$F_{2,24}$=17.39	P=<0.001
3/13/2017	39.4±5c	0.1±0.a1	7.4±3b	$F_{2,23}$=49.62	P=<0.001
3/30/2017	5.8±2b	0.2±0.1a	14.0±4c	$F_{2,24}$=27.09	P=<0.001
4/13/2017	20.2±6c	0.2±0.1a	4.8±1b	$F_{2,24}$=29.43	P=<0.001
4/27/2017	29.6±6c	1.0±0.9a	7.4±3b	$F_{2,24}$=28.17	P=<0.001
5/11/2017	11.4±2b	12.3±7ab	2.0±0.8a	$F_{2,24}$=4.34	P=0.025
5/30/2017	3.6±1a	65.0±55b	11.6±3ab	$F_{2,24}$=3.40	P=0.05
6/12/2017	27.6±8a	66.2±24a	192.6±34b	$F_{2,24}$=8.92	P=0.001
6/26/2017	82.3±13a	57.8±23a	107.4±46a	$F_{2,24}$=1.32	P=0.87
7/11/2017	273.4±36b	47.8±20a	84.0±22a	$F_{2,24}$=15.75	P=<0.001
7/27/2017	47.6±13a	93.0±69a	75.8±27a	$F_{2,24}$=2.93	P=0.073

Chapter 4: Acaricidal Control of Broad Mites in Blackberry, 2016

There are very few treatment options for blackberry growers against the pest broad mite, *Polyphagotarsonemus latus* (Banks). Since 2007 when this mite was reported damaging primocane-fruiting blackberries (*Rubus L. subgenus Rubus*) in Arkansas, is has expanded its damage distribution of blackberries to include multiple other US states and several countries. In the USA, only Agri-Mek® (abamectin) has been registered to control broad mites on caneberries (*Rubus sp.*), with support of data from this study.

During this study, nine compounds: Agri-Mek® (3.5 oz/A), JMS Stylet Oil (1%,2%), M-Pede (1%,2%), Microthiol (10lb/A,15lb/A), Movento 240SC (8oz/A), Penetrate (2%,4%), Quillaja (2%,4%), Trilogy (1%,2%) and Zeal (3oz/A) were used in a lab bioassay to determine their potential for use as control agents of broad mites on caneberries. Leaflets with broad mites were dipped in each solution, dried and placed on a prepared petri dish equipped with moistened sponges, Kimwipes and water. Two percent M-Pede, Microthiol, 2% JMS Stylet Oil, 1% Trilogy and 4% Quillaja formulations had over 80% total mite mortality at 3 DAT; therefore, they could be recommended for use in rotation with Agri-Mek® for *P. latus* control on blackberries.

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Acaricidal Control Of Broad Mites On Blackberry, 2016

Primocane Blackberry: *Rubus* subgenus *Rubus* L., 'Prime-Ark® Freedom"

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Broad Mite (PL): *Polyphagotarsonemus latus* (Banks)

Introduction

Polyphagotarsonemus latus (PL), also known as Broad Mite, is an emerging pest of primocane blackberries in multiple states in the U.S.A. In this study, we evaluated the efficacy of some miticides against PL. Currently, Agri-Mek® SC is the standard and only miticide labeled for blackberries against PL.

Materials and Methods

Blackberry leaf dip petri dish bioassays were used to determine efficacy of several miticides against PL. These bioassays were conducted in the laboratory at the University of Arkansas Southwest Research and Extension Center (SWREC) in Hope, AR. Treatments were arranged in an RCB design with five replicates. On July 25, 2016 (Table 1), and August 25, 2016 (Table 2), PL-infested blackberry leaflets were collected from the first fully opened terminal leaflets of established 'Prime-Ark® Freedom' blackberry plants maintained on the field under a high tunnel at the SWREC. A stereomicroscope was used to scan underside of blackberry leaflets and select those with similar numbers of live PL of all stages. Each mite-infested blackberry leaflet was dipped and swirled for five seconds in a treatment solution, air dried for 30 minutes on paper towels and placed underside up on moistened sponge inside a petri dish. The leaflet petiole was covered with a moistened Kimwipe* (Kimberley-Clark). The sponge was kept moist with distilled water and maintained at 25°C. The nine treatment miticides diluted in distilled water included: Agri-Mek® SC (active ingredient: 8% abamectin; Syngenta Crop Protection, LLC, Greensboro, NC) at rate of 3.5 oz/A (0.256 liters/ha); JMS Stylet Oil (active ingredient: 97% superior grade technical paraffinic mineral oil; JMS Flower Farms, Inc., Vero Beach, FL) at rates of 1% or 2%; M-Pede® (active ingredient: 49% potassium salts of fatty acid; Gowan Co., Yuma, AZ) at rates of 1% or 2%; Microthiol® Disperss® (active ingredient: 80% micronized wettable sulfur; United Phosphorus, Inc., King of Prussia, PA) at rates of 10 lb/A (11.2 kg/ha) or 15 lb/A (16.8 kg/ha); Movento® 240SC (active ingredient: 22.4% spirotetramat; Bayer CropScience LP, Research triangle Park, NC) at rate of 8oz/A (0.58 liters/ha); Penetrate 50 water. nutrition. growth. (w.n.g.) (active ingredient: *Yucca schidigera* plant extract with saponins from surfactant and wetting agent; DPI Global, Porterville, CA) at

rates of 2% or 4%, Quillaja (active ingredient: wood extract of saponin from branches of soap bark tree, *Quillaja saponaria* Molina; DPI Global, Porterville, CA) at rates of 2% or 4%; Trilogy® (active ingredient: 70% clarified hydrophobic extract of neem oil; Certis USA, LLC, Columbia, MD) at rates of 1% or 2%; Zeal™ (active ingredient: 72% etoxazole; Valent U.S.A. Corporation, Walnut Creek, CA) at rate of 3oz/A (0.22 liters/ha); and distilled water check.

At 1 and 3 DAT, a stereomicroscope was used to aid in counting the number of each PL stage within a 2.4 cm^2 leaflet area (1.27 cm x 1.9 cm) grid centered over the midrib on underside of the treated leaflet. Nymph and adult PL were recorded as the numbers of mobile or morbid (no walking or motion within 5 s of observation so presumed dead). The immobile quiescent nymphs were not counted. Percentage mortality data were heteroscedastic requiring arcsine square root transformation prior to ANOVA and use of Tukey's studentized range test (HSD) ($P \leq 0.05$) to distinguish differences among treatment means. Actual non-transformed treatment mean percentage mortality were presented in these tables.

Results

In trial 1 (Table 1), by 1 DAT, Agri-Mek®, both rates of M-Pede, Microthiol and 1% Trilogy all caused similar mortality (> 72%) and were significantly greater than other treatments and water check. By 3 DAT, Agri-Mek®, both rates of M-Pede, Microthiol and 2% JMS Stylet Oil caused 92% or greater mortality. This was larger than, but similar to, both rates of Trilogy (>75%). One percent JMS Stylet Oil (54.8% mortality) was similar to both rates of Trilogy. Except for Agri-Mek®, all non-check treatments achieved highest mortality on 3 DAT. Microthiol 10 lb had higher mortality rates of larvae compared to adult females by 30% and 20% on 1 DAT and 3 DAT (respectively). In trial 2 (Table 2), at both 1 and 3 DAT, Agri-Mek, performed the same as in trail 1. All other treatments except Zeal and the water check exhibited

similar mortality (>60%) at 3 DAT. Four percent Quillaja mortality (>83%), was also statistically similar to Agri-Mek®. Four percent Quillaja caused 23% mortality at 1 DAT but increased to >83% at 3 DAT. For maximum effect, all treatments except Agri-Mek® required 3 DAT. Four percent Quillaja, and both rates of Penetrate increased to >54% mortality from 1 DAT to 3 DAT. Based on these results, 2%M-Pede, Microthiol, 2% JMS Stylet Oil, 1% Trilogy and 4% Quillaja formulations had over 80% total mite mortality at 3 DAT; therefore, they could be recommended for use in rotation with Agri-Mek® for PL control on blackberries.

Acknowledgements

This research was supported by industry gifts of pesticide and research funding.

Table 1. Blackberry leaflet dip laboratory bioassays on toxicity of acaricides against broad mites on blackberries (July 25, 2016).

Treatment/ Formulation	Rate-amt formulation/acre	% Mortality					
		1 DAT			3 DAT		
		Larva	Adult female	Total	Larva	Adult female	Total
Agri-Mek 2SC + Surf-king plus	3.5 fl oz 0.5% v/v (NIS)	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a
M-Pede	2%	91.2a	98.6ab	94.8ab	98.3a	98.0a	97.7a
M-Pede	1%	98.1a	100.0a	98.8ab	96.3a	98.3a	97.0a
Microthiol Disperss	15lb	93.0a	57.5a-d	71.0ba-c	98.3a	93.7ab	97.6a
Microthiol Disperss	10lb	89.1a	59.3b-d	78.0a-c	100.0a	80.0ab	97.5a
Trilogy	2%	69.2ab	63.3a-c	65.5bc	77.2ab	68.7ab	75.5ab
Trilogy	1%	92.0a	87.5a-c	88.6ab	88.8ab	84.2ab	87.1ab
JMS Stylet Oil	2%	73.5ab	75.1a-c	72.7bc	90.6ab	94.1ab	92.7a
JMS Stylet Oil	1%	33.8b	46.4cd	37.6cd	50.6bc	60.1bc	54.8b
Water check	--	15.6b	18.1d	17.5d	11.4c	11.1c	11.0c

NIS = non-ionic surfactant; Means within columns followed by same letter are not significantly different (Tukey's, P>0.05).

Table 2. Blackberry leaflet dip laboratory bioassays on toxicity of acaricides against broad mites on blackberries (August 25, 2016).

Treatment/ Formulation	Rate-amt formulation/acre	% Mortality 1 DAT Larva	1 DAT Adult Female	1 DAT Total	3 DAT Larva	3 DAT Adult Female	3 DAT Total
Agri-Mek 2SC + Surf-king plus	3.5 fl oz 0.5% v/v (NIS)	98.5a	100.0a	99.3a	100.0a	100.0a	100.0a
Quillaja	4%	53.3a-c	24.7b	23.1bc	74.9a	83.1a-c	83.6ab
Quillaja	2%	40.6bc	30.0b	42.5bc	54.5b	94.1ab	65.3bc
Penetrate	4%	9.0bc	25.4b	12.3bc	69.1ab	76.2a-c	68.3bc
Penetrate	2%	11.4bc	25.0b	10.9bc	72.6ab	70.4bc	72.3bc
Movento 240SC + Succeed MSO	8 fl oz + 0.25 v/v	56.9ab	38.0b	50.1b	52.1b	69.7bc	60.1bc
Zeal	3 oz	32.4bc	30.2b	32.5bc	34.3bc	55.6c	47.5cd
Water check	--	5.7bc	4.3b	3.9c	8.3c	15.1d	11.8d

NIS = non-ionic surfactant; Means in same column followed by same letter are not significantly different (Tukey's, P>0.05).

Chapter 5: Efficacy of predatory mites *Amblyseius andersoni* (Chant), *Neoseiulus californicus* (McGregor), *N. cucumeris* (Oudemans) and *N. swirskii* (Athias-Henriot) against *Polyphagotarsonemus latus* (Banks) in Arkansas grown primocane blackberries

Worldwide, predatory mites are utilized as biocontrol agents for many pest insects. To date, none have been tested against broad mites in blackberry plantings. Several predatory Phytoseiid mite species have been reported to control broad mites on various other crops (such as peppers) including *Neoseiulus* (=*Amblyseius*) *swirskii* (Athias-Henriot), *N.* (= *Amblyseius*) *cucumeris* (Oudemans), *A. californicus* (McGregor), *A. herbicolus* (Chant), *A. largoensis* Muma, *Euseius hibisci* (Chant), *E. nicholsi* (Ehara et Lee), *E. ovalis* (Evans), *E. stipulates* (Athias Henriot), *E. victoriensis* (Womeersley), *N. agrestis* (Karg), *N. longispinosus* (Evans), *Typhlodromalus peregrinus* (Muma), *Typhlodromus athiasea* (Proath et Swirski), *N. barkeri* (Hughes) and some Bdeliid mites.

In this chapter, the efficacy of the predatory mites *Amblyseius andersoni* (Chant), *Neoseiulus californicus* (McGregor), *N. cucumeris* (Oudemans) and *N. swirskii* (Athias-Henriot) were tested in 4 locations in Arkansas. The predatory mite efficacy varied by location, with all species demonstrating statistical differences from control plots in at least one location. These preliminary data suggest that predatory mites have great potential to be implemented into an Integrated Pest Management system for broad mites in blackberries.

Acknowledgements

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Chapter 5: Efficacy of predatory mites *Amblyseius andersoni* (Chant), *Neoseiulus californicus* (McGregor), *N. cucumeris* (Oudemans) and *N. swirskii* (Athias-Henriot) against *Polyphagotarsonemus latus* (Banks) in Arkansas grown primocane blackberries

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Abstract

In 2007, the broad mite, *Polyphagotarsonemus latus* (Banks), was discovered damaging primocane-fruiting blackberries (*Rubus L. subgenus Rubus*) in Arkansas. Since then, *P. latus* has been reported damaging blackberries in the US states of Arkansas, California, Illinois, Indiana, Maryland, North Carolina, South Carolina, Tennessee, Virginia, Oklahoma, Pennsylvania, and the countries of China, South Africa and Mexico. Few treatment options are available against *P. latus* in caneberries. Biocontrol with 15 predatory mite species against broad mites on various other crops has been reported. *Amblyseius andersoni, Neoseiulus californicus, N. cucumeris,* and *N. swirskii* were selected to determine their efficacy against broad mites in blackberry fields. Three locations (four fields) within Arkansas were used, treatments were arranged in a randomized complete block design, and the predatory mites were released on 20 May. In all fields, weekly samples of 5 newly expanded terminal leaflets per plot were collected. All life stages of broad mites (egg, larva, female, and male) were counted on the underside of leaflets with a stereomicroscope in a 2.3 cm^2 grid. Predatory mite species efficacy varied by site, with all species having statistical differences from control plots in one site. In 3 of 4 fields, treated plots experienced a broad mite density increase two to three weeks after untreated control plots. This preliminary data suggest that predatory mites have great potential to be implemented into an

Integrated Pest Management system for broad mites in blackberries. Future research should focus delaminating the climate, rates and timing of these biocontrol agents.

Introduction

Worldwide, blackberries are an economically important crop consumed due to their favorable flavor and health benefits (Strik and Thompson 2009). In 2007, the broad mite, *Polyphagotarsonemus latus* (Banks), was discovered damaging primocane-fruiting blackberries (*Rubus L. subgenus Rubus*) in Arkansas (Vincent et al. 2010). Since then, broad mites have emerged as a significant pest of blackberries, causing yield loss by damaging cane terminals, which is the site of berry formation. Currently, blackberry broad mite damage has been reported in the USA in Arkansas, California, Illinois, Indiana, Maryland, North Carolina, South Carolina, Tennessee, Virginia (Johnson 2017 personal communication), Oklahoma (Rebek 2017), Pennsylvania (Demchak and Johnson 2017), and on strawberries in Florida (Renkema et. al. 2017). Additionally, growers in China, South Africa and Mexico have reported the presence of this pest on blackberries (Seagraves 2017 personal communication). In the USA, there are few control methods, and only Agri-Mek® (abamectin) has been registered to control broad mites on caneberries (*Rubus sp.*) (LeFors et al. 2017). Growers need additional methods to control this pest. Predatory mites are used widely in the USA and other countries to control crop pests.

Several Phytoseiid predatory mite species have been reported to control broad mites on various other crops including *Neoseiulus* (=*Amblyseius*) *swirskii* (Athias-Henriot), *N.* (= *Amblyseius*) *cucumeris* (Oudemans), *A. californicus* (McGregor), *A. herbicolus* (Chant), *A. largoensis* Muma, *Euseius hibisci* (Chant), *E. nicholsi* (Ehara et Lee), *E. ovalis* (Evans), *E. stipulates* (Athias Henriot), *E. victoriensis* (Womeersley), *N. agrestis* (Karg), *N. longispinosus* (Evans), *Typhlodromalus peregrinus* (Muma), *Typhlodromus athiasea* (Proath et Swirski), *N. barkeri* (Hughes) and some Bdeliid mites (Shipp and Wang 2003; Weintraub et al. 2003; Weintraub et al. 2007; van Maanen et al. 2010; Calvo et al. 2015; Duarte et al. 2015; Vacante

2016; Lopez et al. 2017; Rodriguez-Cruz et al. 2017). Biological control success resulted when initial releases of predatory mites occurred at low densities of prey (Pickett and Gilstrap 1986; van Maanen et al. 2010). For example, an initial release ratio of 1:20 (released 2 females of *A. swirskii* to 20 broad mites on sweet pepper plants with 10 to 20 leaves) resulted in successful biological control of fewer than 4 broad mites per plant (van Maanen et al. 2010). Therefore, it is probable that some of these species would provide biological control of broad mites in blackberries in Arkansas.

Sulfur has been used as an effective miticide and has demonstrated high mortality of broad mites; but its use is limited because it can cause phytotoxicity when temperatures exceed 32°C and negatively effects natural enemies (Venzon et. al. 2013; LeFors et. al. 2017). Prior to predatory mite release, sulfur (Microthiol Disperss, micronized wettable powder) could be used to knock down broad mite before densities exceed 5 broad mites per leaflet in May and temperatures exceed 32°C.

The objective of this study was to determine the efficacy of May foliar applications of sulfur and/or May releases of predatory mites, *A. andersoni, A. californicus, N. cucumeris,* and *N. swirskii,* to control broad mites in fields of primocane blackberry plantings (*Rubus* L. *subgenus Rubus*).

Materials and Methods

Locations

Several blackberry fields were selected in three different Arkansas locations due to presence of broad mites and damage to terminal leaves. The two fields (named Hilltop and Fence) were used at the University of Arkansas Fruit Substation in Clarksville, AR (35°32’05.2” N 93°24’02.5” W). Both fields contained multiple breeding accessions of primocane

blackberries. Each field had three rows, divided into 3 m plots, with treatments applied to every other plot (buffer in between). A 1 ha commercial field of Prime-Ark® 45 blackberries was used 5near Judsonia, AR (35°22'28.43" N 91°39'47.75" W). This field had five 91 m long rows, 3.9 m apart, and each divided into twelve 7.6 m long plots delimited by trellis posts with treatments applied to every other plot (buffer in between). A field of Prime-Ark® Freedom blackberries inside a plastic covered high tunnel (24 m long x 7.6 m wide x 3.6 m high) was used at the University of Arkansas Southwest Research and Extension Center (SWREC) in Hope, AR (33°42'34.38" N 93°33'35.25" W). This field had three 23 m long rows, 2.4 m apart, and each divided into three, 7.6 m long plots delimited by trellis posts with treatments applied to each plot separated by a 1 m plant-free buffer.

Treatments

The following predatory mite species released in this study were all purchased from Rincon-Vitova Insectaries, Inc., Ventura, CA including: *A. swirskii* (100 Swirski-Mite Plus sachets each with 250 mites/sachet, hanger, bran and food mite packaged by Koppert Biological Systems, B.V. The Netherlands); *A. cucumeris* (260 Amblyline™ sachets with 1,000 mites/sachet, hanger, bran and food mites packaged by Bioline AgroSciences Ltd., Little Clacton, Essex); *A. andersoni* (100 Bioline® Anderline™ sachets with hanger, bran and food mites, each with 250 mites/sachet packaged by Bioline Agrosciences Ltd., Little Clacton, England); and *A. californicus* (1000 mites/bottle with bran, Rincon-Vitova Insectaries, Inc., Ventura, CA). These paper sachets had a mite exit hole and a hanger to hang sachet on the plant. Each sachet contained predatory mites, bran and a storage food mite that sustained predatory mite reproduction and slow release for several weeks.

On May 20, 2016, predatory mite species *N. cucumeris*, *A. andersoni*, *N. swirskii* were released at rates of 1000, 250, and 250 mites per sachet, respectively, with one sachet were hung one per plant beneath leaves in the upper canopy on each of the 12 central primocanes in each plot. A bottle of 1000 *A. californicus* predatory mites and bran were shaken directly onto leaves in the upper canopy in each plot after lightly misting leaves with water using a spray bottle. The same release rates were used in each field. All treatments were arranged in randomized complete blocks.

For each species of predatory mite, one sachet or bottle was opened and inspected to verify viability. Vouchers specimens of each predatory mite species were transferred from sachet or bottle to a drop of Hoyer's on slides and preserved as described by Krantz (2009).

In Clarksville, treatments included (three replicates): 1) untreated control; and separate releases of predatory mites 2) *N. cucumeris*; 3) *A. andersoni*; 4) *N. swirskii*; and 5) *N. californicus* each on May 20, 2016.

Near Judsonia, treatments included (5 replicates): 1) untreated control; 2) foliar applications of Microthiol (sulfur) at a rate of 16.8 kg/ha (15 lb/acre) on May 7 and 20, 2016; and separate releases of predatory mites on May 20, 2016 of 3) *N. cucumeris*; and 4) *N. swirskii*.

In Hope, treatments included (3 replicates each): 1) untreated control; 2) foliar applications of Microthiol Disperss at a rate of 16.8 kg/ha on May 5 and 23, 2016; and 3) foliar application of Microthiol Disperss at a rate of 16.8 kg/ha applied May 5, 2016 followed with release of *N. cucumeris* on May 20, 2016.

To quantify broad mite populations, weekly samples of five newly expanded terminal leaflets were collected from the central portion of each plot. The five leaflets per plot was determined used based on two years of prior sampling in Arkansas (Donn Johnson, 2016

Personal communication). All stages of broad mites (egg, larva, female, male) on the underside of each leaflet within a 2.3 cm^2 grid centered on midrib and starting at the petiole were counted with the aid of a Zeiss Stemi 2000-c stereomicroscope (Carl Zeiss Microscopy, LLC, Peabody, MA) and recorded. Predatory mites found on leaflet samples were collected and stored in 90% Ethanol for future identification. Voucher slides were made of selected samples and adults were identified to species (*P. latus*) as described in Krantz 2009.

Statistics

Data were log (x+1) transformed to best fit assumptions of linearity and homogeneity of variances. Analysis was completed with an ANOVA Type III test, LSMEANS and Tukey's studentized range test (HSD) with $P<0.05$ (R Development Core Team 2017). Each field was analyzed independently due to location effect.

Results

On 10 June, the Hilltop plots in Clarksville, the untreated control had significantly more ($F_{4,10}=4.19$; $P = 0.002$) broad mites per plot than did the three plots receiving a release of predatory mites, *A. andersoni, N. californicus, and N. swirskii* (Fig. 1). On 24 June, the Clarksville Fence plots had significantly more ($F_{4,10}=4.6$; $P= 0.001$) broad mites per plot in the untreated control than plots where *N. californicus* were released (Table 1) (Fig. 1).

In Judsonia, AR, on 16 June, the untreated control plots had significantly ($F_{3,16}=6.4$; $P=0.0002$) more broad mites than did plots sprayed first with Microthiol or had a release of *N. cucumeris* (Table 1). On 10 June, broad mite counts began increasing in the untreated control plots (Fig. 1). It was not until 30 June, that broad mite counts increased in plots treated with sulfur, *N. swirskii* or *N. cucumeris,* when count were similar to that in the untreated control plots (Fig. 1).

In Hope, AR on 13 and 21 June, the untreated control plots had significantly ($F_{2,6}$=13.5; P =0.001) more broad mites per plot than did treated plots of sulfur and sulfur with *N. cucumeris* (Table 1). After 21 June, both treatments (sulfur and sulfur with *N. cucumeris*) experienced increases in broad mite counts that were similar to counts in the untreated plots (Fig. 1).

Discussion

In at least one site, the individual release of *N. swirskii, A. andersoni, N. cucumeris* or *N. californicus* each maintained broad mite densities below that recorded in untreated plots for several weeks. One month after releases were made, the broad mite densities increased in all blackberry fields. This may imply that predatory mites need to be released earlier when broad mites are first detected in the field in order to provide adequate biological control.

The efficacy of these predatory mites could be affected by seasonal changes in broad mite densities caused by dispersal (unknown mechanism). By late-May, broad mite reproduction had begun with the appearance of adult males carrying resting nymphs and evidence of oviposition. Damaging broad mite densities were restricted to a few plants in a blackberry field. By mid-June, there was a more even distribution of damaging densities of broad mites across the field (described in Chapter 3). All four predatory mite species released in at least one location in these studies successfully maintained low counts of broad mites for a few weeks before counts increased and became similar to those in the untreated control plots. Therefore, each predatory mite species has the potential to provide biological control of broad mites in blackberries.

There may also be an effect of climate (temperature, rain and relative humidity) since the field inside the high tunnel at Hope was warmer and drier than the open fields at Clarksville whereas the Judsonia field was under a shade cloth that provided more hours of high relative humidity and dew on the blackberry plants than the other dryer fields. Future research should be

conducted in larger plots to overcome problem of broad mite dispersal in order to compare efficacy against broad mites of different release rates (predator: prey ratios) of each of these predatory mite species under different climates. Different application methods could also be evaluated including blower release of predatory mites (Opit et al. 2005) and targeting aggregation areas before field wide release or using additional release dates in succession to achieve control until after fruiting.

There is concern that purchased predatory mites may not be the species requested. In this study, voucher mite specimens were collected from the *N. cucumeris* sachets and later identified as a different species *Typhlodromalus jucundus* (Muma) with the aid of Gilberto de Moraes. This is of interest because this species thought to be *N. cucumeris* reduced broad mite numbers per plot.

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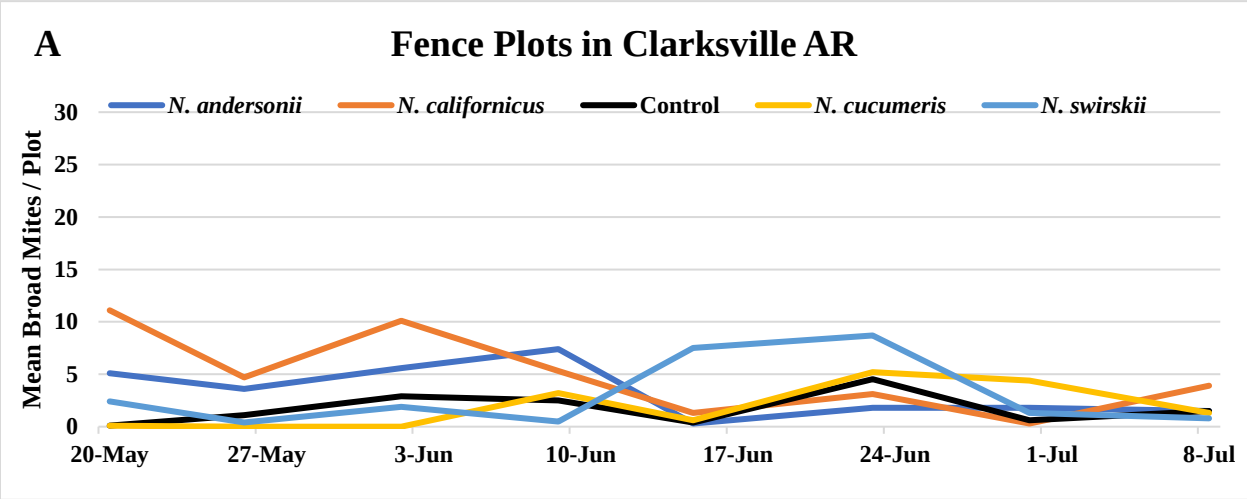
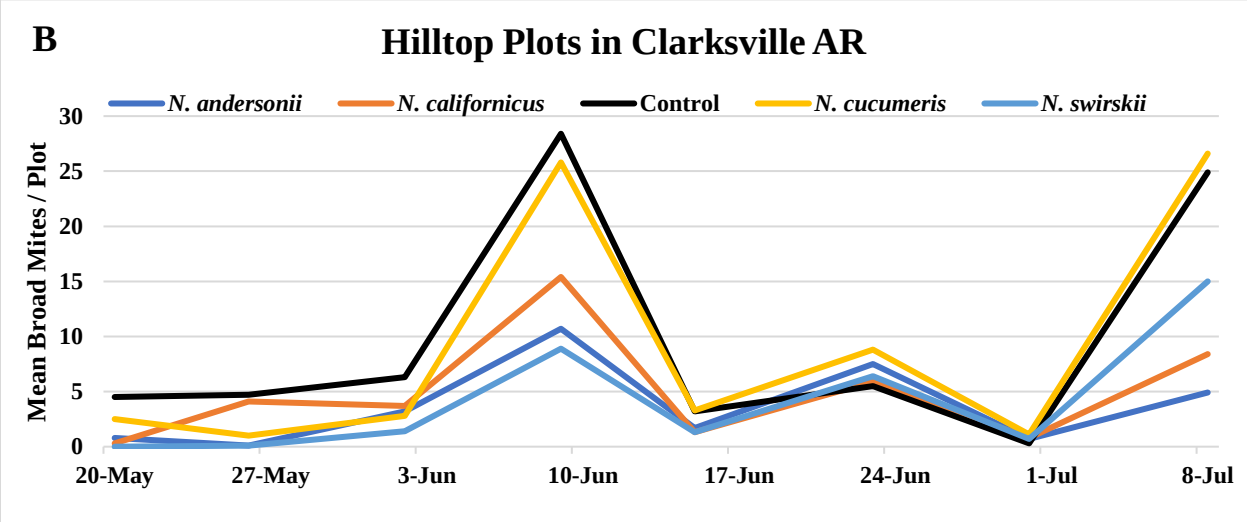

A
Fence Plots in Clarksville AR
N. andersonii
N. californicus
Control
N. cucumeris
N. swirskii
Mean Broad Mites / Plot
30
25
20
15
10
5
0
20-May
27-May
3-Jun
10-Jun
17-Jun
24-Jun
1-Jul
8-Jul


B
Hilltop Plots in Clarksville AR
N. andersonii
N. californicus
Control
N. cucumeris
N. swirskii
Mean Broad Mites / Plot
30
25
20
15
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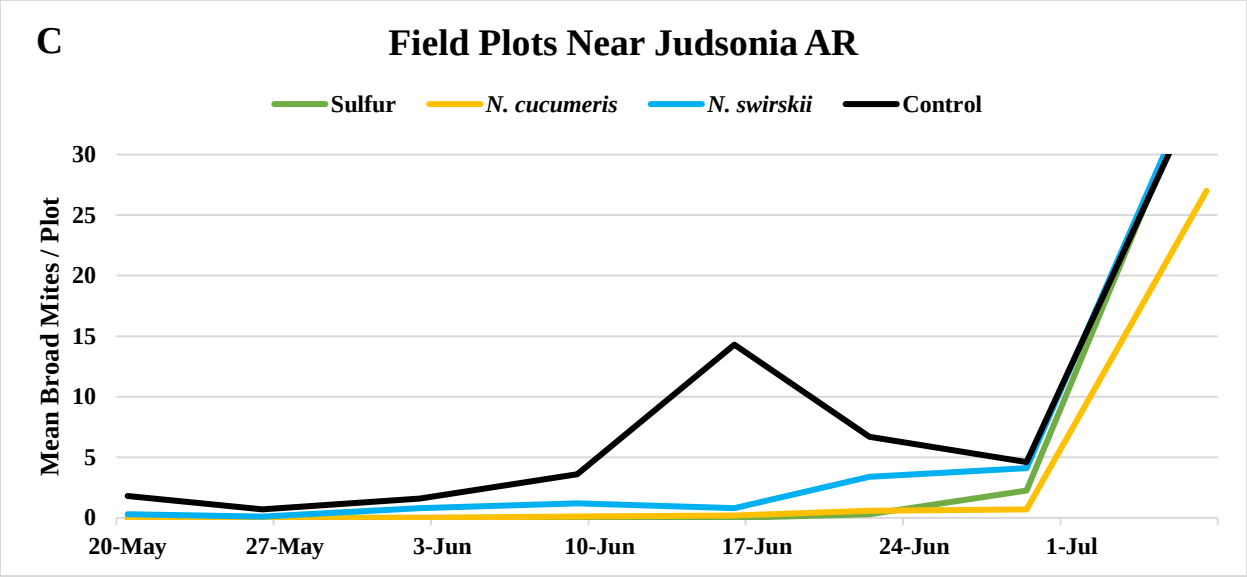
5
0
20-May
27-May
3-Jun
10-Jun
17-Jun
24-Jun
1-Jul
8-Jul



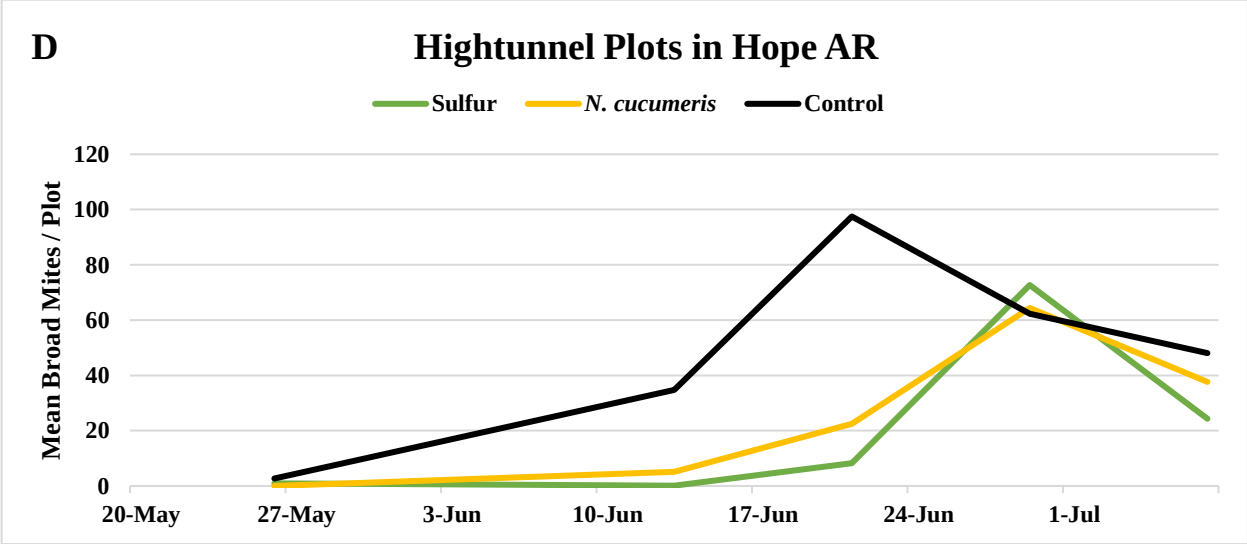


Figure 1: Weekly mean numbers of broad mites per treatment plot in four Arkansas blackberry fields in 2016: A) Hill plots and B) Fence plots both at the Fruit Station in Clarksville, AR (N = 4); C) plot near Judsonia, AR (N = 5); and D) plot inside a plastic covered high tunnel at the SW Research and Extension Center in Hope (N = 3). Each predatory mite species was released alone on May 20. Microthiol (sulfur) alone was applied to foliage on May 7 and 20 in the Judsonia plots and May 5 and 23 in the Hope plots. Another treatment plot only at Hope was Microthiol applied on May 5 followed by release of *N. cucumeris* on May 20.

Table 1: Season total mean numbers (±SE) of broad mites per treatment plot in four Arkansas blackberry fields in 2016: Fence (N = 3) and Hill (N = 4) fields at the Fruit Station in Clarksville, included untreated control and separate releases of four predatory mite species on 20 May; near Judsonia (N = 5) included untreated control, foliar application of sulfur alone on 7 and 20 May and predatory mite releases of *N. cucumeris* or *N. swirskii* on 20 May. C) SW Research and Extension Center in Hope, AR (N = 3) included foliar application of sulfur on 5 and 23 May alone and with predatory mite releases of *N. cucumeris* on 20 May (12 sachets per plot). Log+1 transformed data were used for ANOVA and LSMEANS, raw means are reported below.

	Clarksville fields			
Treatments	Fence	Hill	Judsonia	Hope
Control	1.7±0.5b	9.7±1.5 a	8.8±1.7 a	49.1±6.9 a
Sulfur	-	-	5.3±1.4 b	21.2±5.2 b
Sulfur + *N. cucumeris*	-	-	-	25.9±6.1 b
A. andersoni	3.2±0.7ab	3.7±0.8b	-	-
N. californicus	5.0±1.0a	5.0±1.0b	-	-
N. cucumeris	1.8±0.6b	8.2±1.9ab	3.7±0.9 b	-
N. swirskii	2.9±0.7ab	4.2±1.1b	5.3±1.1 ab	-
F value	$F_{4,10}$=4.19	$F_{4,10}$=4.6	$F_{3,16}$=6.4	$F_{2,6}$=13.5
P value	0.002	0.001	0.0002	0.001

Mean values in same column with same lower case letters are not significantly different (Tukey's studentized range test (HSD), P>0.05)

Chapter 6: Conclusion

Broad mites are an increasingly important pest of blackberries in the USA and other countries. Prior to this study, there was little information on population dynamics of broad mites in blackberry fields and in the USA limited options available to control this pest.

During this study, a sampling technique, the Water Sugar-Water method (WSW) was developed for samples collected from blackberry fields to estimate seasonal changes in densities of *P. latus* on blackberry plants. It was determined that the WSW method extracted >95% of mites from a Tullgren funnel sample stored in alcohol for different periods of time, and effectively separated mites from sample debris making mite identification and counting easier. This method worked well for extracting mites from all sample types including blackberry leaves, leaf litter and soil of differing textures. Taxa from 12 different families or superfamilies of mites were collected and it is expected this method can be applied to other animal groups for ecological studies and describing seasonal changes in population dynamics.

The seasonal phenology and changes in broad mite densities per blackberry leaf, leaf litter and soil sample were described for blackberry plantings in open fields and inside high tunnels. In Arkansas, the minimum daily temperatures dropped below 0°C from 20 December 2016 to 8 February 2017. This resulted in broad mite numbers per blackberry leaf dropping from 300 in early-December to near zero by 23 February. Over this same period, there were < 7 broad mites per soil sample, but leaf litter samples sustained above 200 broad mites per sample. After 23 February, counts from leaf litter samples dropped to and remained below 25 broad mites per sample until early-June. This indicated that broad mites were mostly surviving winter cold in the leaf litter under the blackberry plants. In late-May, broad mites began to reproduce as noted by increasing numbers on developing terminal blackberry leaves. This initial distribution was

aggregated with hot spots within the field showing the first sign of terminal leaf bronzing and curling. From 30 May to 27 July, broad mites appeared to be dispersing as counts increased and remained above 50 broad mites per soil (the only time of year that broad mites appeared in soil samples) and leaf litter sample. In 12 days, the numbers of broad mites per leaf per plot had increased and the distribution of mites and foliar damage become more uniform across the whole field. This provided evidence that broad mites were dispersing by some unknown means. These research blackberry plots did not have whiteflies but did have thrips that could disperse broad mites by phoresy (Palevsky et al. 2001). Broad mite aerial dispersion was not found in a preliminary study described by Palevsky et al. (2001). Starting in May, broad mites may be carried down the blackberry row by plant-to-plant, hand soft tip pruning of primocane terminals infested with broad mites. More research is needed to determine how broad mites are dispersing in blackberries.

Bioassays found that Microthiol, Trilogy, M-Pede, JMS stylet oil, and Quillaja caused >80% mortality 3 days after treatment. These compounds should be further evaluated in fields to determine optimal rates but could be recommended for use in rotation with Agri-Mek®.

Of the four predator mites used for field efficacy trials in 2016, all species, *N. cucumeris, N. californicus, N. swirskii* and *A. andersoni* had significant fewer broad mites per plot than the control plots. However, the results were not consistent across all fields. This is presumably due to varying field conditions such as rain causing sachets to fall to ground or warmer climate in the more southern plots in SW Arkansas. More studies are needed to illustrate which predatory mite species does best against broad mites when released in blackberries produced in different climatic conditions in open fields and high tunnels in Arkansas.

Findings from this study were used to compose an extension fact sheet to inform blackberry growers identify broad mites understand its biology, and to sample for and manage broad mites. In Arkansas, growers need to begin in early-May to scout blackberry fields weekly. Initially, look for a "hot spot" in the field that is showing the first signs of bronzing and curling up or down of developing terminal leaves. Count and record the number of broad mites on the underside of each of ten leaflets collected from newest fully developed terminal leaves from canes adjacent to and including the damaged cane. If the average count exceeds five broad mites per leaflet, then you need to treat this infested area with a recommended miticide. Sulfur and JMS stylet oil can be applied if temperatures are below 32°C, Agri-Mek can be applied (only two applications per season) in accordance with the label and preharvest intervals. This mite is found during the winter in leaf litter, and to a lesser extent under blackberry bud scales. Therefore, cultural controls such as winter removal of blackberry leaf litter and tip pruning (once canes are dormant) may help reduce populations in the upcoming season (yet to be demonstrated as effective). The predatory mites *Neoseiulus cucumeris*, *N. californicus*, *N. swirskii* and *Amblyseius andersoni* could be released in mid-May and more released 3 weeks later to delay broad mite densities increasing to damaging levels.

Future research should focus on specific conditions required for each species of predatory mite, field application trials of compounds that showed to be promising in the lab bioassays, and focus on the causes of aggregation and subsequent dispersal could lead to more treatment options. In addition, while not tested in this study there is much evidence to suggest that both entomopathogenic fungi and bacteria could be assayed and added to the rotation for control of broad mites on blackberries.