

**The natural history and predator complex of the native pine bark adelgid (*Pineus strobi*)
Hartig in southwestern Virginia**

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**ABSTRACT
(ACADEMIC)**

The pine bark adelgid, *Pineus strobi* (Hemiptera: Adelgidae) is a native herbivore of eastern white pine, *Pinus strobus* (Pinales: Pinaceae), in eastern North America. It is a sessile insect that settles on *P. strobus* and inserts its stylet bundle to feed on the tree's phloem. Although *P. strobi* is not considered a serious pest, it shares its range with the invasive hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae). Predators introduced as biological control agents of *A. tsugae* interact with *P. strobi* and its native predators, including *Laricobius rubidus* LeConte (Coleoptera: Derodontidae). Prior to this study, little work had been done to document the phenology or predators of *P. strobi*, particularly in its southern range. In the present study, the phenology of *P. strobi* is reported in southwestern Virginia. Patterns in overwintering population dynamics varied notably from those described from this species' northern range. The number of annual generations could not be measured due to overlap following two distinct spring generations. Adult body size varied seasonally and was greatest in the spring. Variation between observations from the northern and southern ranges of *P. strobi* indicate phenological plasticity that informs biological control efforts and offers insight into implication of climatic effects on population dynamics of this and related species. Arthropod predators associated with *P. strobi* in forests of southwest Virginia were collected during a two-year survey. Morphology and DNA barcoding were used for identification. Species of predators found included: *Laricobius rubidus*

(Coleoptera: Derodontidae), a native adelgid specialist, and two species from the dipteran family Chamaemyiidae, *Leucopis piniperda* Malloch and *L. argenticollis* Zetterstedt, which are adelgid specialists. Members of the families Cecidomyiidae, Coccinellidae, Chrysopidae, Hemerobiidae, and Syrphidae were also recovered. Most diverse were the Cecidomyiidae, with 15 different species inferred from their DNA barcodes. Additional work was performed to quantify supercooling points of *L. rubidus* collected from November – December 2016. These will be compared to those of other *Laricobius* species in a parallel study. Knowledge of this predator complex is beneficial to describing *P. strobi* ecology, and also with regard to potential biological control of invasive adelgids in the same region.

The natural history and predator complex of the native pine bark adelgid *Pineus strobi* (Hartig) in southwestern Virginia

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**ABSTRACT
(GENERAL AUDIENCE)**

The pine bark adelgid, *Pineus strobi* (Hemiptera: Adelgidae) is a native herbivore of eastern white pine, *Pinus strobus* (Pinales: Pinaceae), in eastern North America. It is a sedentary insect that settles on *P. strobus* bark, branches, and needle bases where it inserts its mouthparts to feed on the tree's fluid nutrients. Even when *P. strobi* populations are dense, it does not harm the tree and is not considered a serious pest. It does, however, share its range with the invasive hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae). Predators introduced as biological control agents of *A. tsugae* also interact with *P. strobi* and its native predators, including *Laricobius rubidus* LeConte (Coleoptera: Derodontidae). Prior to the current study, little work had been done to document the natural history or predators of *P. strobi*, particularly in its southern range, both of which are relevant to the management of other invasive adelgid species found there. In the present study, the natural history of *P. strobi* is reported in southwestern Virginia. Patterns in the frequencies of various life stages present during the winter varied notably from those described from this species' northern range. The number of annual generations could not be accurately measured due to the degree of overlap following two distinct spring generations. Adult body size varied seasonally and was greatest in the spring. Variation between observations from the northern and southern ranges of *P. strobi* indicates that its natural history can vary with the climate, which not only informs biological control efforts,

but also offers insight into implication of climatic effects on the population dynamics of this and related species. This study also describes arthropod predators associated with the pine bark adelgid in forests of southwest Virginia found during a two-year survey. Physical characteristics and molecular techniques were used in identification. Species of predators found include: *Laricobius rubidus* (Coleoptera: Derodontidae), a native adelgid specialist, and two species from the family Chamaemyiidae, *Leucopis piniperda* Malloch (Diptera: Chamaemyiidae) and *L. argenticollis* Zetterstedt (Diptera: Chamaemyiidae), which are known adelgid specialists. Members of the families Cecidomyiidae, Coccinellidae, Chrysopidae, Hemerobiidae, and Syrphidae were also recovered. Most diverse were the Cecidomyiidae, with 15 different species genetically identified. Additional work was performed to quantify the bodily freezing points of *L. rubidus* collected from November – December 2016. These will be compared to those of related biological control agents in a parallel study. Knowledge of this predator complex is beneficial to describing *P. strobi* ecology, and also with regard to potential biological control of other invasive adelgids in the same region.

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ATTRIBUTION

Several colleagues aided in the writing and research behind the following chapters presented as part of this dissertation. A brief description of their contributions is included here.

Chapter 2: Phenology of the pine bark adelgid, *Pineus strobi* (Hemiptera: Adelgidae), in white pine forests of southwestern Virginia. Published in *Environmental Entomology*: nvx161, <https://doi.org/10.1093/ee/nvx161>.

Scott M. Salom, PhD is a professor of forest entomology in the Department of Entomology at Virginia Tech. Dr. Salom was second author on this paper, and assisted in experimental design, conduct, and data analysis. Dr Salom was also the principal investigator for the grant supporting the research, and contributed editorial comments.

Thomas P. Kuhar, PhD is a professor of vegetable crop entomology in the Department of Entomology at Virginia Tech. Dr. Kuhar was a co-author on this paper, and assisted in data analysis and contributed editorial comments.

Chapter 3: Predators associated with the pine bark adelgid, *Pineus strobi*, in forests of southwestern Virginia.

Nathan P. Havill, PhD is a research entomologist for the USDA Forest Service. Dr. Havill conducted DNA barcoding research and analysis on insect samples referenced in this study and contributed editorial comments.

Scott M. Salom, PhD (Department of Entomology, Virginia Tech) assisted in experimental design, conduct, and contributed editorial comments.

Thomas P. Kuhar, PhD (Department of Entomology, Virginia Tech) assisted in experimental design and contributed editorial comments.

Chapter 4: Supercooling points of *Laricobius rubidus* in southwestern Virginia.

Scott M. Salom, PhD (Department of Entomology, Virginia Tech) assisted in experimental design, conduct, and contributed editorial comments.

Thomas P. Kuhar, PhD (Department of Entomology, Virginia Tech) assisted in experimental design and contributed editorial comments.

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CHAPTER ONE

Literature Review

Pine Bark Adelgid

Members of the family Adelgidae (Order Hemiptera) are small, soft-bodied insects with simple morphology and are endemic to temperate and boreal environments of the Northern Hemisphere. They are phytophagous and highly host-specific. Adelgids are most closely related to insects of the families Aphidae and Phylloxeridae; like them, adelgids have characteristically complex, multigenerational, polymorphic life cycles involving cyclical parthenogenesis (Havill and Footitt 2007).

The pine bark adelgid (PBA), *Pineus strobi* (Hartig), was the first of its genus to be studied in North America. However, its original description was somewhat confused, so early accounts may be flawed (Annand 1928, Doane 1961). It is native to eastern North America, its range matching that of the eastern white pine, *Pinus strobus* L., the main host plant of this insect (Annand 1928, Doane 1961, Steffan 1970, Carter and Barson 1973, Stekolshchikov and Novgorodova 2012). *Pineus strobi* has been introduced to Europe, as an exotic pest (Mattson et al. 2007), and also to the state of Washington near Tacoma, where it was recently found feeding on western white pine (*Pinus monticola*) (Douglas ex D. Don) (Darr 2017). The pine bark adelgid feeds on bark, branches, and base of needles on *P. strobus*, inserting its stylets, which measure approximately 1.7 mm in length, intercellularly far enough into the tree to reach the outer tissues of its live phloem (Clark and Brown 1960, Doane 1961, Raske and Hodson 1964). When populations are dense, the trunk of an infested tree appears to be covered by a white cottony mat (Doane 1961). Those adelgids settling at the base of needles prefer needle clusters

on each year's new growth. On seedlings, *P. strobi* is restricted to settling at needle bases or on the bark in areas at or just below ground level. The reason for this seems to be that when trees are young their canopy is insufficient to provide necessary shade for the adelgid, which is intolerant of exposure to direct sunlight. On lower branches; however, the insect finds shade and cooler temperatures, preferring a temperature range of 15-20°C, with > 30°C unfavorable to survival. Likewise, on trees having one side consistently exposed to the sun, *P. strobi* will be more often found on the shaded side; no such pattern is observed on trees located away from direct sunlight (Raske and Hodson 1964).

Pineus strobi has been observed to have five generations per year with an average lifespan of 35 days, allowing it to reach moderately high population levels at times (Raske and Hodson 1964). This is the case even in the presence of predators, although adelgid populations are often reduced significantly in the summer when predators are most active (Wilson 1938, Clark and Brown 1960, Raske and Hodson 1964). However, even when *P. strobi* densities are high, little or no discernable injury is inflicted to trees, even over the course of years (Clark and Brown 1960).

Typical of members of the family Adelgidae, *P. strobi* has a complex life cycle involving multiple generations, in which different forms display varying morphology and alternate host plants, migrating between a spruce species and another conifer. In the case of *P. strobi*, black spruce, *Picea mariana* (Mill.), is thought to be the most likely primary host (although sexual reproduction has never been observed), and *P. strobus* is the secondary host where parthenogenic reproduction occurs (Annand 1928, Balch and Underwood 1950, Raske and Hodson 1964, Havill and Footitt 2007). The pine bark adelgid seems to be functionally anholocyclic, in that no sexual generation is known to exist (Havill and Footitt 2007). There are two apterous parthenogenic forms, the progrediens present in summer and the sistens present throughout the year. These

generations overlap and can be found simultaneously. They are difficult to tell apart, as their morphology is similar, though progrediens tend to be slightly larger (adult progrediens measure 0.7-1.2 mm long × 0.5-0.7 mm wide vs. adult sistens 0.6-1.1 mm long × 0.5-0.7 mm wide) (Raske and Hodson 1964). However, progrediens have one more nymphal instar than sistens, molting four times rather than three (Raske and Hodson 1964). Though all life stages are present throughout the year, *P. strobi* most often overwinters as a third instar in very cold locations (Doane 1961, Raske and Hodson 1964), becoming active when temperatures reach approximately 10°C, and maturing in the spring. From the eggs that these adelgids lay in the spring (Clark and Brown 1960, Doane 1961, Raske and Hodson 1964), some neosistens will hatch, but also during certain years, there will be a small subset of sexuparae – a winged form that fly to *P. mariana* in an attempt at sexual reproduction (Annand 1928, Doane 1961, Raske and Hodson 1964, Carter and Barson 1973). Once on the spruce, the sexuparae settle on needles of opening buds and lay eggs on these needles and then die. After egg hatch, the crawlers die after migrating to the base of needles on new growth; none have been observed to survive or reproduce (Raske and Hodson 1964). When PBA flight has been observed, it has been found to occur most often during the afternoon in June, likely due to favorably warm temperatures (Carter and Barson 1973).

Because apterous forms of *P. strobi* are the most common and relevant, most phenological information known of this insect focuses on them. Details of the various life stages of the pine bark adelgid follows.

Adult *P. strobi* are present year-round (Doane 1961, Raske and Hodson 1964). They are approximately 1 mm long and 0.6 mm wide (detailed measurements given previously), soft-bodied, and tear drop shaped with legs and antennae not easily visible. Adults begin laying eggs

4-6 days after eclosion, initially laying only 1 egg every day or two. After about a week, oviposition rate peaks at about 6 eggs per day, gradually decreasing thereafter. Usually 40-50 eggs are laid by a single female. Females lay eggs for 20-30 days; therefore, eggs laid earlier have hatched before oviposition has concluded (Raske and Hodson 1964).

Eggs are uniform in size, measuring 0.3 mm long \times 0.1 mm wide. Their color ranges from an initial milky yellowish brown that lightens after half an hour to orange as the embryo develops. A dark red color is indicative of a nonviable egg. Eggs develop for 7-15 days at spring temperatures, and for 6-8 days during warmer summer temperatures (Raske and Hodson 1964).

Each instar lasts an average of 5 days (range 4-8 days) (Raske and Hodson 1964). The first instars that emerge from eggs are referred to as crawlers, and their morphology is quite distinct from other instars, having a longer, narrower body with longer, more visible legs and antennae. This is the only stage of apterous adelgid capable of dispersal. They may move of their own accord by crawling or are carried farther away by wind or animals, such as birds (Doane 1961, Raske and Hodson 1964, Carter and Barson 1973, Havill and Foottit 2007). Crawlers exhibit positive phototaxis and are capable of moving at a rate of 1.3 cm per minute at 22°C. They have been found capable of living 10 h without feeding, and so should be able to cover approximately 8 m on a tree before choosing a spot to feed where they will remain for the duration of their lifetime. Though immature insects must extract their stylets from the tree trunk in order to molt, they nevertheless remain in the same location; those that have been observed to wander away have not survived (Raske and Hodson 1964). Crawlers usually settle in protected areas, under bark or old wool or within the stipule of the needle bundle. When densities are high, crawlers often congregate, but can be found singly when populations are lower. Once settled, crawlers begin to produce their filamentous, waxy “wool”. Adelgids produce this substance from special

wax glands on their dorsum, likely for use in physical and chemical protection (McClure 1989, Jones et al. 2014). There are two subsequent nymphal instars following the crawler stage in the case of *sistens* and three more instars in *progreiens* individuals. This cycle continues throughout the year with no resting stage during the summer (Raske and Hodson 1964).

The pine bark adelgid rarely reaches damaging levels. This is likely due to multiple factors, but it is evident that adelgid numbers are kept in check, at least in part, by predators. Known predators of the pine bark adelgid include: *Laricobius rubidus* LeConte (Coleoptera: Derodontidae), *Chilocorus bivulnerus* (Mulsant) (Coleoptera: Coccinellidae), *Microweisa* spp. (Coleoptera: Coccinellidae), *Scymnus suturalis* Thunberg (Coleoptera: Coccinellidae), *Leucopis simplex* (Loew) (Diptera: Chamaemyiidae), *L. obscura* (Haliday) (Diptera: Chamaemyiidae), *L. pinicola* (Malloch) (Diptera: Chamaemyiidae), *Hemerobius stigma* (Stephens) (Neuroptera: Hemerobiidae), *Wesmaelius concinnus* (Stephens) (Neuroptera: Hemerobiidae), *Lestodiplosis pini* (Barnes) (Diptera: Cecidomyiidae), and *Tetrapphelps* spp. (Heteroptera: Anthocoridae) (Wilson 1938, Raske and Hodson 1964, Sluss and Foote 1973, Montgomery and Lyon 1995a, McAvoy et al. 2007). Of these, all are native to eastern North America with the exception of *L. obscura*.

Laricobius rubidus

Perhaps the most important predator of *P. strobi* is *Laricobius rubidus*, the only member of its genus native to eastern North America (Lawrence and Hlavac 1979). It feeds on all life stages of this adelgid, and its range matches that of *P. strobi* (and hence that of *Pinus strobus*) (Brown 1944, Clark and Brown 1960, Montgomery and Lyon 1995a). *Laricobius rubidus* is found on

trees of all sizes and is usually found together with *P. strobi* (Clark and Brown 1960). The known phenology of this predator matches that of its prey (Clark and Brown 1960, Zilahi-Balogh et al. 2005, Mausel et al. 2008). The beetles break hibernal diapause in early spring, and adults are active from March – June (Clark and Brown 1960, Zilahi-Balogh et al. 2005). They have been observed to be univoltine in northern regions of their range. *Laricobius rubidus* has a sex ratio of 1:1 and mating begins soon after adult emergence and continues through May. Eggs are laid during April and May; they are deposited singly within the wool of the adelgid ovisac, oviposition itself taking 15 minutes. After they are laid, eggs are covered with a mucilaginous secretion to ensure adhesion to wool. They measure 0.4 mm long × 0.29 mm wide and are a creamy yellow color with a smooth and shiny chorion (Clark and Brown 1960). Eggs hatch within a week, and larvae mature through 4 larval instars while feeding on *P. strobi* adults, eggs, and nymphs (Clark and Brown 1960, Mausel et al. 2008). The hallmarks indicative of *L. rubidus* predation are tattered and dislodged wool and desiccated adelgid carcasses beneath. *Laricobius* beetles feed by piercing adelgids with their mandibles and feeding on their hemolymph (Mausel et al. 2008). Though it has not been directly observed, it is thought that late instar *L. rubidus* larvae migrate to the base of the tree and pupate below ground in June (Clark and Brown 1960).

Laricobius rubidus is closely related to other congeners that used for biological control of invasive adelgid species. All *Laricobius* species with known biology specialize on members of the family Adelgidae (Lawrence and Hlavac 1979). Though larvae may be indistinguishable morphologically, adults of *L. rubidus* can be distinguished from other species. Distinctive features include red-hued elytra and unique structure of male genitalia. Specifically, the lateral lobes of the aedeagus are obliquely truncate, and the angle of the parameres are diagnostic (Brown 1944, Mausel et al. 2008, Leschen 2011, Havill et al. 2012). In the case that larvae need

to be identified without being reared to the adult stage, they can be distinguished using molecular techniques involving diagnostic nucleotide sequence differences using cytochrome oxidase I (COI) DNA barcoding sequences (Davis et al. 2011). Being so closely related to other *Laricobius* species used for biological control may indeed be cause for concern, or at least warrant deeper investigation. This topic will be discussed in further detail.

Laricobius nigrinus* and Biological Control of the Hemlock Woolly Adelgid *Adelges tsugae

The hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae), is a devastating pest of eastern hemlock, *Tsuga canadensis* (L.) and Carolina hemlock, *Tsuga caroliniana* Engelman in the eastern U.S. *Adelges tsugae* is native to East Asia and western North America, and was accidentally introduced to the eastern U.S. from Japan (McClure 1989, Havill et al. 2006). It was first found in its introduced range in 1951 in Richmond, VA and has since spread throughout much of the Appalachian region (Souto et al. 1996, EDDMapS 2017). *Adelges tsugae* is bivoltine and parthenogenic, with a complex holocyclic multigenerational life cycle (McClure 1989, Havill and Footitt 2007). The hemlock woolly adelgid is active from the fall through spring, aestivating over the summer (McClure 1989, Lamb et al. 2007, Mausel et al. 2008). Like *P. strobi*, *A. tsugae* has two parthenogenic forms, sistens and progrediens, on its secondary host. Sistens are present for most of the year from July through April; progrediens are found April through June (McClure 1989). *Adelges tsugae* eggs are laid from late winter through early spring, and insects develop through four instars, beginning with a mobile crawler stage (McClure 1989, Mausel et al. 2008).

Like many invasive species, *A. tsugae* is damaging in the eastern United States for multiple reasons: it has no predator community to contend with in its new range, and its new host plant

does not have the inherent resistance as do the hosts that have co-evolved with it (Montgomery and Lyon 1995b, Wallace and Hain 2000, Lagalante et al. 2006). Insecticides, particularly neonicotinoids, are effective against *A. tsugae*. However, application at the landscape level is not feasible for economic and logistical reasons (many hemlock stands are in remote areas that are not easily accessible) (McClure 1992). Thus, much hope for control of this invasive pest relies upon effective biological control. In *A. tsugae*'s western North American range, one of its most important specialist predators is *Laricobius nigrinus* Fender (Coleoptera: Derodontidae) (Kohler et al. 2008ab). Since 2003 these beetles have been released and successfully established throughout the introduced range of the hemlock woolly adelgid (Flowers et al. 2005, Lamb et al. 2006, Mausel et al. 2010). *Laricobius nigrinus* is a well-suited biological control agent for use against *A. tsugae*: it prefers to feed on *A. tsugae* and can develop only on this prey, has a life cycle synchronized with *A. tsugae*, consumes prey in relatively large numbers, and can survive and flourish in plant hardiness zones 6a and above (Zilahi-Balogh et al. 2002, 2003a, 2003b, Mausel et al. 2010, 2011, Story et al. 2012).

Hybridization of *L. rubidus* and *L. nigrinus*

Although *L. rubidus* is a specialized host of *P. strobi*, it is occasionally found on other adelgid hosts, including *A. tsugae*. In fact, *L. rubidus* seems to be adapting to the increased presence of this introduced prey source and is frequently found on hemlock (Montgomery and Lyon 1995b, Zilahi-Balogh et al. 2005, Mausel et al. 2008, Story et al. 2012). There may even be evidence that *L. rubidus* is more active during the winter than previously thought in the South, meaning it is active when *A. tsugae* are present and developing. Additionally, *L. rubidus* has been found to be able to complete development on *A. tsugae* (Zilahi-Balogh et al. 2005).

Association of both *Laricobius* beetles with *A. tsugae* and the fact that eastern white pine and hemlock stands are frequently mixed provides opportunity for interaction between *L. rubidus* and *L. nigrinus* (Davis et al. 2011). These species have been observed mating with one another in the field. The offspring produced from such pairings are fertile hybrids and there is indication of advanced hybrid generations. This indicates the likelihood of broadly occurring hybridization between these species (Havill et al. 2012). Hybridization cannot be determined molecularly using the same DNA barcoding methods sufficient to determine pure *L. rubidus* from *L. nigrinus*, because it relies upon mitochondrial DNA, which will only track an individual's maternal lineage (Davis et al. 2011). Instead, a technique was developed using 6 microsatellite loci found to be diagnostic for these species, and thus straightforward to interpret, in addition to mitochondrial COI haplotypes. This method has been determined to be reasonably accurate, though in generations following a single backcross event, hybridization may go undetected due to the low number of markers (Havill et al. 2012). Investigation into *L. rubidus* X *L. nigrinus* hybridization indicates widespread introgression strongly asymmetrical towards *L. nigrinus* on hemlock, which could affect the host preference of hybrids towards *A. tsugae*. Hybridization with *L. rubidus* may have positive implications for establishment of *L. nigrinus*, alleviating Allee effects and supporting adaptation to the novel environment of the eastern U.S. While there was initial concern that this event may harm the integrity of *L. rubidus* as a species or the predator/prey balance between it and *P. strobi* (Havill et al. 2012), research suggests it will not. Fischer et al. (2015) showed evidence that, due to each predator's respective prey preference, displacement of *L. rubidus* by *L. nigrinus* on *P. strobus* is unlikely. However, an unforeseen impact of this biological control program is nevertheless possible and may not be apparent for a

relatively long time, as is sometimes the case in such programs involving forest systems (Reilly and Elder 2014).

Rationale and Objectives

Though *P. strobi* is not a serious pest, causing only periodic aesthetic damage to Christmas trees, it is important that its ecology in southwestern Virginia be documented. Variation in its phenology between southern and previously studied northern ranges will provide valuable insight into the roles of abiotic factors on its phenology. Such information may even be extrapolated and applied to others of the Adelgidae family, some of which are of greater economic significance. Impact of abiotic environmental inputs is increasingly critical to recognize at this time of quickly shifting climactic conditions.

Also important is elucidating the role of *P. strobi* as a food source for predators in the forest ecosystem. Reporting the complex of predators associated with this native species provides knowledge of the forest food web, which is intrinsically valuable. At a time of rapid loss of species to extinction, it is necessary to appreciate the role played by even the tiniest of animals in this ecosystem.

Beyond the inherent value of understanding the ecology of an under-studied species, *P. strobi* may serve as an alternate food source for biological control agents released to manage *A. tsugae*. Knowing the phenology of this native adelgid often found at the same sites as *A. tsugae* will inform these programs, especially in the event of high winter mortality of *A. tsugae* or early emergence from dormancy of predators in rearing facilities. Additionally, knowledge of predator-prey interactions involving *P. strobi* will allow for recognition of disruption resulting

from introduction of biological control agents intended for *A. tsugae* management. It is important that as much information be gathered as possible to prevent displacement of native species through competition, hybridization, or other unintentional means.

For these purposes, it is the **objective of this research** to document the seasonal phenology of *P. strobi* and its associated predators in the forests of southwestern VA. Specific attention is paid to *L. rubidus* as a specialist predator.

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CHAPTER TWO

Phenology of the pine bark adelgid, *Pineus strobi*, in white pine forests of southwestern Virginia

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Abstract

The pine bark adelgid, *Pineus strobi* (Hemiptera: Adelgidae) is a native herbivore of eastern white pine, *Pinus strobus* (Pinales: Pinaceae), in eastern North America. *Pineus strobi* does not appear to have any dominant overwintering lifestage in southwest Virginia, as it does in its northern range. Eggs can be found consistently from late March through early December and may be produced sporadically later throughout the winter during warm periods. Two distinct generations were observed in the spring, after which life stage frequencies overlapped. Adult body size varied seasonally and was greatest in the spring. The present study constitutes the first recording of phenological details of the *P. strobi* in its southern range, informing biological control efforts aimed at closely related invasive pests. The phenological plasticity observed between northern and southern *P. strobi* populations provides insight into the potential effects of climate on the population dynamics of this and related species.

Introduction

The pine bark adelgid, *Pineus strobi* (Hartig) (Hemiptera: Adelgidae) is small, soft-bodied, and phytophagous, primarily feeding on eastern white pine, *Pinus strobus* L. (Doane 1961, Havill and Footitt 2007). Both species are native to eastern North America and share the same range distribution (Annand 1928), although *P. strobi* has also been identified as an introduced exotic species in Croatia (Matosevic and Zivkovic 2013) and in the Pacific northwestern US on *Pinus monticola* (Douglas ex D. Don) (Darr 2017). *Pineus strobi* feeds by inserting its stylets into the tree, reaching live phloem tissue, and extracting photosynthate. An individual becomes anchored to the tree after choosing a feeding site, and so is immobile for the majority of its life. There is only brief opportunity for dispersal during the crawler stage, and though it can cover only a short distance on its own, it may travel within and between trees or stands if carried by wind or passing animals, as with other adelgids (McClure 1990). *Pineus strobi* may settle at the base of needles, on stems, branches, or the trunk of the tree (Doane 1961, Raske and Hodson 1964). After feeding commences, *P. strobi* begin to secrete a white, presumably protective “wool” (Raske and Hodson 1964). Though populations may become quite dense, giving the infested portion of the host a white matted appearance, *P. strobi* does not appear to impact tree health (Clark and Brown 1960).

Pineus strobi becomes active at temperatures above 10°C, at which point adults will begin to lay eggs. Raske and Hodson (1964) reported that oviposition began in the spring in Minnesota, one of the colder portions of its range. Temperatures of 15-20°C are optimal, whereas those above 30°C appear unfavorable for development. At optimum temperatures, eggs hatch 6-8 days following oviposition, but may take up to 15 days if temperatures are consistently low. In Minnesota, adults are reported to oviposit for a period of 20-30 days, laying a maximum of 6

eggs per day and producing up to 50 eggs per female in her lifetime. *Pineus strobi* has three instars: a mobile crawler stage and two subsequent nymphal stages, with each instar lasting 4-8 days (Raske and Hodson 1964).

All individuals are female and reproduce parthenogenically. *Pineus strobi* is anholocyclic (Havill and Footit 2007), and most are apterous. The majority are of the sistentes generation, however, a small subset of individuals produced in the spring may in some instances, perhaps due to high population density, develop to become winged sexuparae that will fly to black spruce, *Picea mariana* (Mill.) Britton, Sterns & Poggenburg. There, they oviposit on needles adjacent to opening buds, but no offspring survive past the first instar (Marchal 1913). Though these eggs may hatch, the offspring have never been observed to survive beyond the first instar. Sexuparae develop alongside the progrediens, another apterous, morphologically distinct generation found in the springtime in less abundance than the sistens (Raske and Hodson 1964). *Pineus strobi* is not known to undergo diapause, and is observed to have overlapping generations. In its northern range, the predominant overwintering stage is reported to be the 3rd instar, but all life stages can be found throughout the year (Doane 1961, Raske and Hodson 1964).

Pineus strobi is considered an occasional pest on ornamental trees and Christmas trees for aesthetic reasons, but it is rarely bothersome enough to require control measures. However, its ecological role as an alternate food source for predators of invasive adelgid species makes its patterns of seasonal life stage abundance relevant to biological control efforts in forest ecosystems. The hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae), and the balsam woolly adelgid, *Adelges piceae* (Ratzeburg) (Hemiptera: Adelgidae) are two invasive adelgid species found in the native range of *P. strobi*, both of which have had significant ecological and economic impact following their introduction to eastern North America (Jenkins

2003, McClure 1991). *Scymnus suturalis* Thunberg (Coleoptera: Coccinellidae) is an established predator in the eastern US that feeds readily on all *A. tsugae* lifestages, but to survive it requires a source of adelgid eggs to feed on during the summer. The presence of *P. strobi* eggs during this time allows *S. suturalis* to function as a biological control of *A. tsugae* (Lyon and Montgomery 1995, Montgomery and Lyon 1995). Likewise, there are a host of generalist predators, such as those belonging to the Cecidomyiidae, Syrphidae, Chloropidae, and Hemerobiidae families, exploiting *P. strobi* in its native range that also feed on *A. picea* and *A. tsugae* (Montgomery and Lyon 1995).

Although *P. strobi* was the first of its genus to be studied in the United States, relatively little is known about it. Most information regarding its natural history pertains only to populations found in Minnesota near the far northwest corner of its range, with a brief account of its biology in Connecticut. Until the present study, *P. strobi* phenology has not been described in its southern range. Any differences observed in this species' patterns of seasonal population structure between northern and southern aspects of its native range is ultimately informative to understanding potential impacts of climate-driven abiotic factors on the population dynamics of other adelgid species, several of which have significant environmental and economic impact in North America. The present study reports the seasonal phenology of *P. strobi* including changes in life stage frequency and body size, as well as a narrative describing trends observed in distribution of this species in forests of southwestern Virginia.

Materials and Methods

Three research sites in southwestern Virginia were identified and used. Sites included: Peak Creek, at Gatewood Park and Reservoir, Pulaski, VA, N37.06217° W80.83633°; Hussy Mountain, in Mount Rogers National Recreation Area, Speedwell, VA, N36.77223° W81.17521°; Dismal Falls, in George Washington and Jefferson National Forest, Giles, VA, N37.19263° W80.89230°. The Dismal Falls site lies in plant hardiness zone 6b, while both Hussy Mountain and Peak Creek are in zone 6a (USDA Plant Hardiness Zone Map 2012). These sites were chosen based upon several criteria, including a consistently measurable density of *P. strobi* on *Pinus strobus* and year-round accessibility by vehicle. Data collection began February 11, 2014 and concluded November 6, 2015.

Each site was visited biweekly during the sampling period, or as close to that timeframe as possible if prevented by inclement weather. Sampling was limited to parts of trees accessible from the ground. Mature white pines, saplings, and seedlings were sampled opportunistically when adequate *P. strobi* densities were observed. Because of the relative scarcity of this native insect, it was not feasible to implement a structured sampling scheme with regard to tree age class or number of samples collected per individual tree. Observations were recorded regarding trends observed in *P. strobi* distribution both within and among host trees. *Pinus strobus* branch tips and bark infested with *P. strobi* were collected and refrigerated until examination under a dissecting microscope. The first 100 live *P. strobi* specimens found on infested material from each site and sampling date were removed and placed on a flat surface to be measured (length, width, and head width) using 75X magnification. Individuals were classified by life stage based on morphological features, and eggs found appearing viable were counted; non-viable eggs were characterized by either their bright red coloration or shriveled appearance. Branch tip processing

ceased once 100 specimens of non-egg life stages were recovered. Because of their uniform size, only a subset of 100 eggs collected across sites was measured. In some instances of low density, fewer than 100 individuals could be found on infested material collected. In these cases, all specimens found were processed. The tendency of *P. strobi* to settle in close proximity to one another, often with many individuals sheltering in the same bark crevice under a continuous segment of “wool”, prohibited some methods of assessment. For example, it was often unclear how many maternal adults contributed to eggs encountered; this is why eggs were recorded individually rather than as clusters. Also, cast exoskeletons could not reliably be matched to a single insect and only morphological evidence for life stage classification could be used.

Mean daily temperature at each site was determined by averaging the daily high and low temperatures collected from weather stations using BioSIM software (Régnière 1996) Because average daily temperatures were similar across sites, data from all three sites were averaged.

Statistical Analysis

All statistical analyses were performed using JMP Pro 11.0 (SAS 2013). Life stage frequencies, expressed as proportions of the sample population, from all field sites and across both years were pooled, grouped by season, blocked by site and year, and averaged. Data were normalized using an arcsine transformation of proportions; a constant of 0.000001 was added to all proportions to remove zeroes from the data set (Zar 2013). Least squares analysis was used to test for significant differences among life stages within seasons. Means were compared using Tukey’s HSD at $P < 0.05$.

Body size measurements (length, width, and head width) of all non-egg individuals were averaged within life stages and standard deviation of this mean was calculated. Measurements of *P. strobi* collected during the spring were excluded due to the likely presence of progrediens, which are not reliably distinguishable from sistens but are known to be larger in size than the more abundant sistens individuals and to have an additional instar. Adult head widths from all sites and both years were pooled, grouped by season, and averaged for 2014 and 2015. A one-way ANOVA was used to test for significant differences in seasonal adult head width. Means were compared using Tukey's HSD at $P < 0.05$.

Results

Pineus strobi were most often found in sheltered locations – within needle sheaths; in crevices existing on trunk, branch, or stem surfaces; beneath portions of aged wool; etc. – but could occasionally be seen on exposed surfaces. It is possible in such situations that small shelter structures were dislodged during collection and transportation to the lab. At low densities, most typical of the forested sites sampled, *P. strobi* were most frequently found on new growth or at the base of needles. Only in less common instances of higher densities were they found on the woody portions of mature branches or smooth bark of tree trunks. *Pineus strobi* populations appeared to have very spotty distribution within the forest and were difficult to find in significant density over most of the area of the forest. There were, however, small areas identified, up to approximately 0.4 ha in size, that consistently supported relatively high *P. strobi* densities for several years. The locations of these populations were scattered, and few common features were shared among them topographically or otherwise. One feature they did share was that they

typically existed near the edges of roads or footpaths, although this observation could have been biased by the fact that scouting often occurred along roads or trails.

Pineus strobi begins oviposition in late March or early April, as average daily temperatures consistently approached or exceeded 10°C (**Fig. 2.1**). A dramatic peak in egg frequency occurred at all sites in both years in early April, usually with a second peak in mid-May (**Fig. 2.1a**). Egg frequency peaks were followed chronologically by peaks in crawler frequency (**Fig. 2.1b**). After the second peak in crawler frequency, patterns in life stage frequencies became difficult to discern. During this time, crawlers were concentrated on the terminal buds of their hosts, often nestled within bud scales. Consistent oviposition ceased in December when average daily temperatures remained below 10°C, although it did occur sporadically following short periods of warmer winter days (**Fig. 2.1**). Overwintering populations were represented by all non-egg life stages with no one stage clearly greater in abundance (**Fig. 2.1**).

Mean seasonal percentages of each life stage were analyzed, pooling results from all sites and both years, and while there were abundant overwintering third instars, they were not significantly dominant over second instars (**Fig. 2.3**, $F_{(7,117)} = 42.6$, $p < 0.0001$). Overwintering adults were observed at a significantly lesser frequency than third instars but constituted similar percentages of the sample population as first and second instars. Eggs, though present at low frequencies, were the least commonly observed overwintering life stage. In the spring, eggs were the most abundant life stage, followed by adults, which were significantly more abundant than second instars. Otherwise, a significantly greater percentage of the spring populations were composed of adults than second instars (**Fig. 2.3**, $F_{(7,177)} = 32.7$, $p < 0.0001$). Summer populations were also dominated by eggs, with all other life stages occurring at very similar frequencies (**Fig 2.3**, $F_{(7,217)} = 65.0$, $p < 0.0001$). In the fall, the proportions shifted, and eggs were present at lesser

frequencies than any other life stage except crawlers. Instead, third instars made up the greatest percentage of the sample population, with crawlers, first instars, and adults all found at similar frequencies (**Fig. 2.3**, $F_{(7, 72)} = 7.4$, $p < 0.0001$).

Mean *P. strobi* body lengths, widths, and head widths are reported in **Table 2.1**. To avoid inadvertent inclusion of data from members of the progrediens generation, measurements here exclude those taken from specimens collected in the spring, with the exception of eggs. Data show that there considerable overlap in body size between various life stages, and that these parameters could not be used as a distinguishing feature. Head capsule measurements tended to be a more reliable indicator of actual body size because of their greater sclerotization. When mean head capsule widths of adults collected seasonally across the sampling period were compared, those collected in the spring of both 2014 ($F_{(3, 1870)} = 131.0$, $p < 0.0001$) and 2015 ($F_{(3, 1385)} = 155.7$, $p < 0.0001$) were significantly greater than at any other time of year (**Fig. 2.4**). Also in 2014, mean adult head capsule widths in summer were greater than they were in the winter, and in 2015 they were greater than mean fall and winter head capsule widths.

Discussion

At all sites and across both years, *P. strobi* eggs suddenly explode in number early in April after having been virtually absent during the winter. Spikes in egg frequencies were consistently followed by elevated numbers of crawlers collected. This phenomenon was also reported by Raske and Hodson (1964) in Minnesota, where it was referred to as “the big hatch”. They observed the onset of *P. strobi* oviposition on 24 April, 1960. Observations of this study suggest eggs are laid earlier in southwestern Virginia, where they were first seen as early as 1 April in 2015 at the Hussy Mountain site. Onset of oviposition coincided with maintenance of daily mean temperatures above 10°C for at least several days (**Fig. 2.2**). It is important to note, because sites were visited biweekly, it is likely that in some cases, eggs were actually present prior to the date recorded. A second increase in egg and subsequent crawler frequency in mid-May or early June was also observed. Thereafter, although eggs were found continually through early December, in no cases were there discernable spikes in life stage frequencies for the duration of the year, likely due to the overlapping of *P. strobi* generations. (**Fig. 2.1**). This contrasts with the results from Raske and Hodson (1964), who were able to determine voltinism of this species due to highly synchronous generations. It seems likely that discrete generations were not recognizable in the current study because the long oviposition period of maternal *P. strobi* caused staggered hatching dates and subsequent development of offspring. The same phenomena, however, was also described by those authors, who reported oviposition periods to last up to 30 days. Thus, there may be some other factor influencing life stage frequency patterns that differs between northern and southern ranges.

In this study, seasonal trends in *P. strobi* frequency, indicative of its biology in its southern range of southwestern Virginia, were documented and compared to previous reports from its northern range in Minnesota. Spring and summer populations were dominated by eggs; this was when the majority of reproduction took place. All life stages were present in fall of both years. All life stages were present during the winter, though eggs were infrequent, and late instars and adults were more highly represented (**Fig. 2.2**). This is, in part, consistent with previous reports from data collected in *P. strobi*'s northern range. However, Raske and Hodson (1964) describe highly synchronized generations in the spring and summer, contrasted to the extreme overlap observed in the current study that rendered voltinism of *P. strobi* unmeasurable. The former study also found 90 percent of the overwintering population to be third instars with no presence of eggs whatsoever. This may reflect a quiescent response to the extreme cold experienced during Minnesota winters that is not reflected in *P. strobi*'s more temperate southern range. A more homogenous life stage structure during the winter within northern populations may be what lead to greater synchronization among generations through the spring and summer. Beyond complicating attempts to identify discrete *P. strobi* generations, this discrepancy may have broader ecological impacts. For instance, a more heterogeneous population structure may provide a more consistently optimal source of food for predators that may prefer to consume a specific adelgid life stage. This may help to sustain native and introduced predator populations, some of which may alternatively feed on other invasive adelgid species sharing *P. strobi*'s range.

This study is the first to report the mean body dimensions of each life stage of *P. strobi* (**Table 2.1**). Head capsule width was the most reliable measure of overall body size, presumably because the head is more strongly sclerotized and not subject to becoming misshapen as are other dimensions of this soft-bodied insect. Observed seasonal increases in adult head width may be

due to the occurrence of a progrediens generation. *P. strobi* were reported to produce a modest progrediens generation in the summer (Raske and Hodson 1964). Individuals of this generation were found to be larger than those of the sistens generation and undergo an additional instar. They are, however, difficult to discern from members of the sistens generation, with which they are present concurrently, due to extensive morphological intermediates between instars and generations. Data collected in this study support the presence of a progrediens generation in the southern range of *P. strobi*. Following the conclusion of this study, the presence of sexuparae was confirmed when winged adults were collected on 28 April, 2016 in beat sheet samples taken at Dismal Falls. Sexuparae were not, however, observed during collection of phenological data.

An increase in the frequency of a certain life stage, especially an early stage such as egg or crawler, could be interpreted to indicate the occurrence of a new generation of *P. strobi*. In some instances, identifying the number of peaks in a given life stage over the course of a year can be used to determine the number of generations an insect undergoes annually. New generations are more easily recognized soon after eggs are laid in the spring. However, as the year goes on the overlapping of generations obscures this pattern and makes the identification of generations difficult. Two discrete peaks in the frequency of eggs collected in April were, however, observed; this was consistent across all 3 field sites and both years (**Fig. 2.1**). Tracking individual insects throughout the year would be useful in providing definitive evidence of number of *P. strobi* generations per year, though this may be prohibitively difficult due to the size and nature of this species. Alternatively, the construction of a degree day model for *P. strobi* could estimate the number of annual generations in any given location using historical weather data.

Our findings are valuable as baseline biological data of an understudied species. It may prove useful if *P. strobi* is found outside its native range, especially if management is required. Additionally, observations on the phenological adaptations of *P. strobi* to variations in environmental conditions may provide insight into predicting outcomes of introductions of other adelgid species to novel territory. An understanding of *P. strobi* phenology could also contribute to biological control programs to control invasive adelgids in its range, such as the hemlock woolly adelgid, *Adelges tsugae*, and the balsam woolly adelgid, *Adelges piceae*. Biological control agents of *A. tsugae*, such as *Sasajiscymnus tsugae* Sasaji and McClure (Coleoptera: Coccinellidae), *Laricobius* spp. (Coleoptera: Derodontidae), *Scymnus coniferarum* Crotch (Coleoptera: Coccinellidae), *Scymnus suturalis*, or *Leucopis* spp. (Chamaemyiidae: Diptera), may require an alternate food source when *A. tsugae* goes dormant in the summer or disappear temporarily due to extreme cold temperature events. They may potentially utilize *P. strobi* populations, which are often found in the same ecosystems. All these agents have been shown to feed on *P. strobi* in the absence of their preferred host (Butin et al. 2004, Zilahi-Balogh et al. 2002, Grubin et al. 2011, Darr 2017, Montgomery and Lyon 1995). Additionally, although no specialist predators have been identified that have a significant impact on *A. piceae*, generalists in the *P. strobi* predator complex are likely to feed on it as well.

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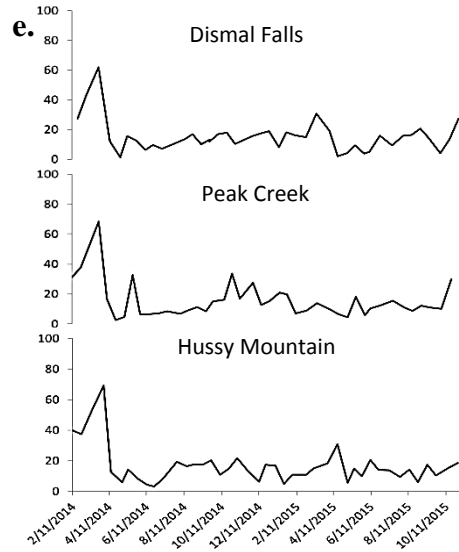
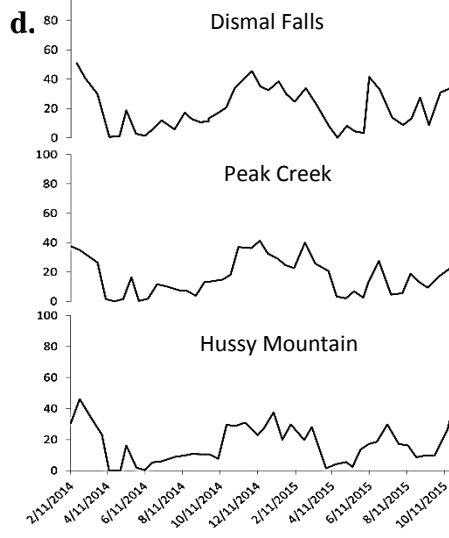
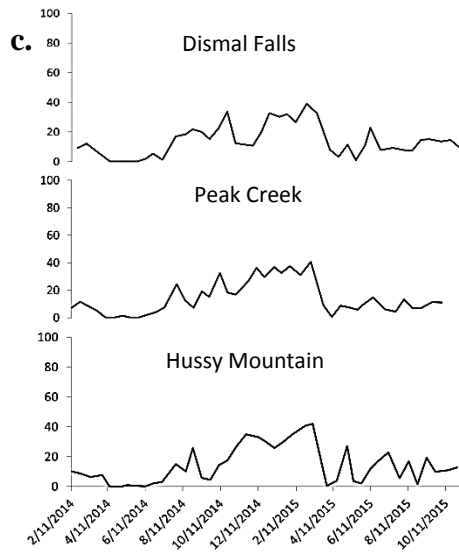
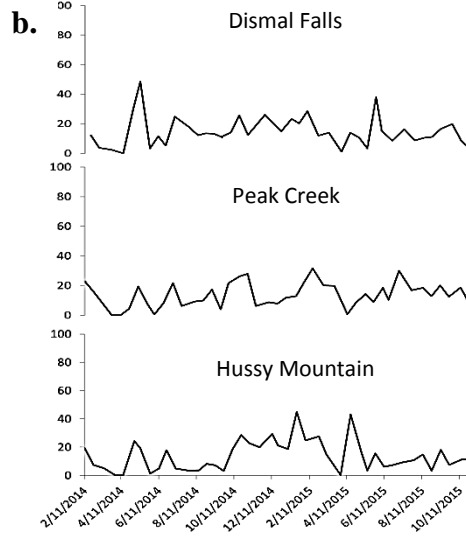
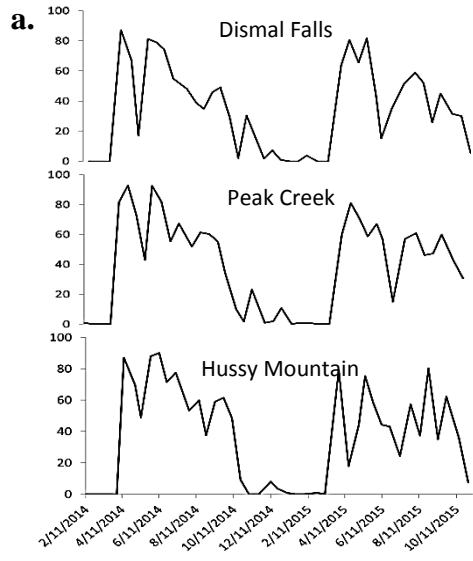
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Tables and Figures

Table 2.1. Mean body length, body width, and head width of *P. strobi* life stages collected in southwest Virginia during 2014 and 2015. Measurements of non-egg life stages collected during the spring are excluded.

Life Stage	Body Length mean \pm std. dev. (mm)	Body Width mean \pm std. dev. (mm)	Head Width mean \pm std. dev. (mm)
Egg	0.27 \pm 0.02	0.14 \pm 0.01	---
Crawler	0.32 \pm 0.05	0.19 \pm 0.03	0.15 \pm 0.04
2 nd Instar	0.38 \pm 0.05	0.27 \pm 0.10	0.23 \pm 0.06
3 rd Instar	0.44 \pm 0.10	0.33 \pm 0.04	0.29 \pm 0.06
Adult	0.53 \pm 0.09	0.42 \pm 0.05	0.34 \pm 0.04

Proportion of Adelgid Sample Population



Date

Figure 2.1. Frequency of egg (a), crawler (b), N₂ (c), N₃ (d), and adult (e) life stages of *P. strobi* shown as proportion of total sample population collected at each site in southwest Virginia on each date across the sampling period.

Average Mean Daily Temperature

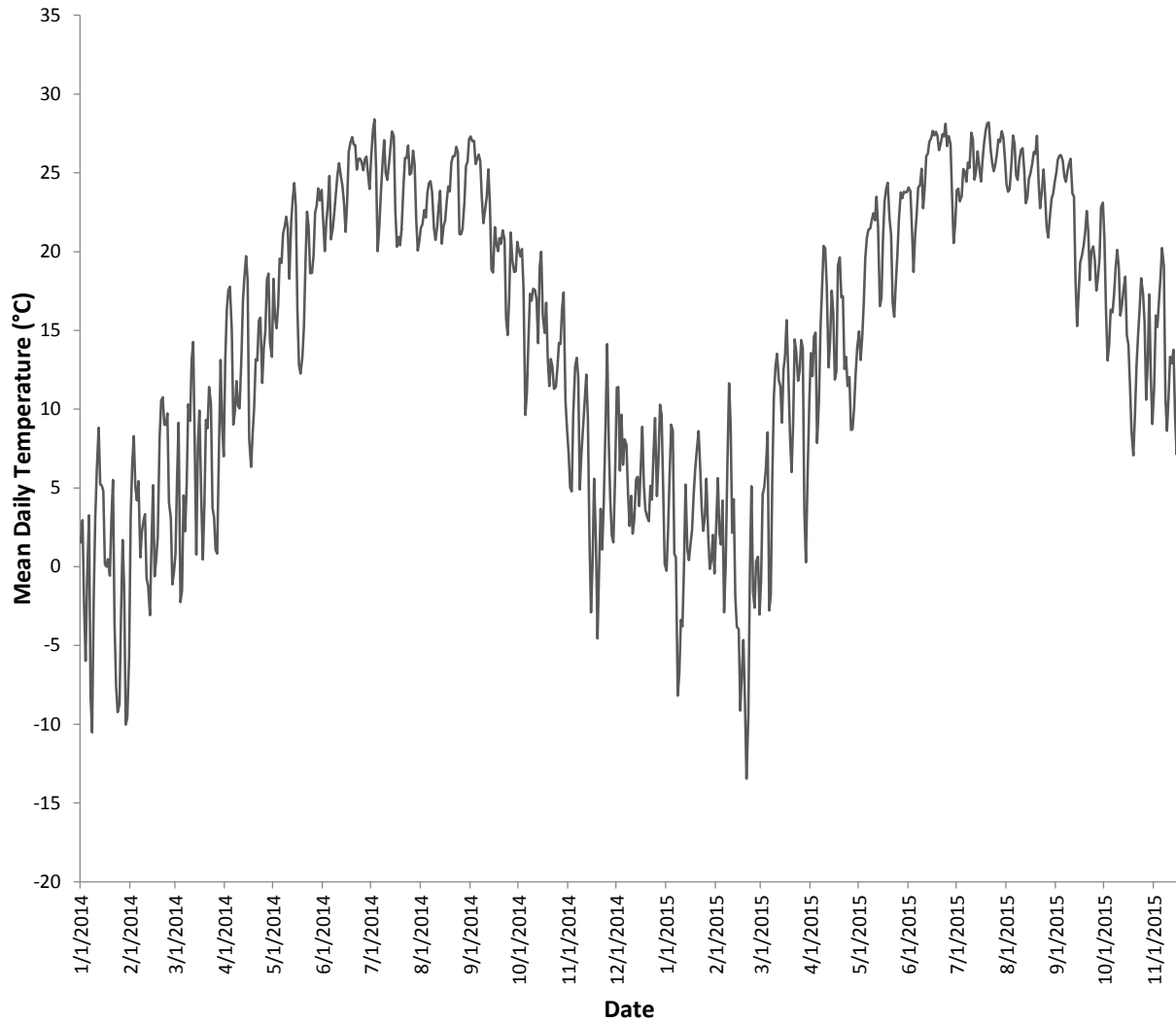


Figure 2.2. Mean daily temperatures (°C) averaged across *P. strobi* sample sites (Peak Creek, Hussy Mountain, and Dismal Falls) in southwest Virginia over sampling period. Temperature data from each site collected using BioSIM software (Régnière 1996).

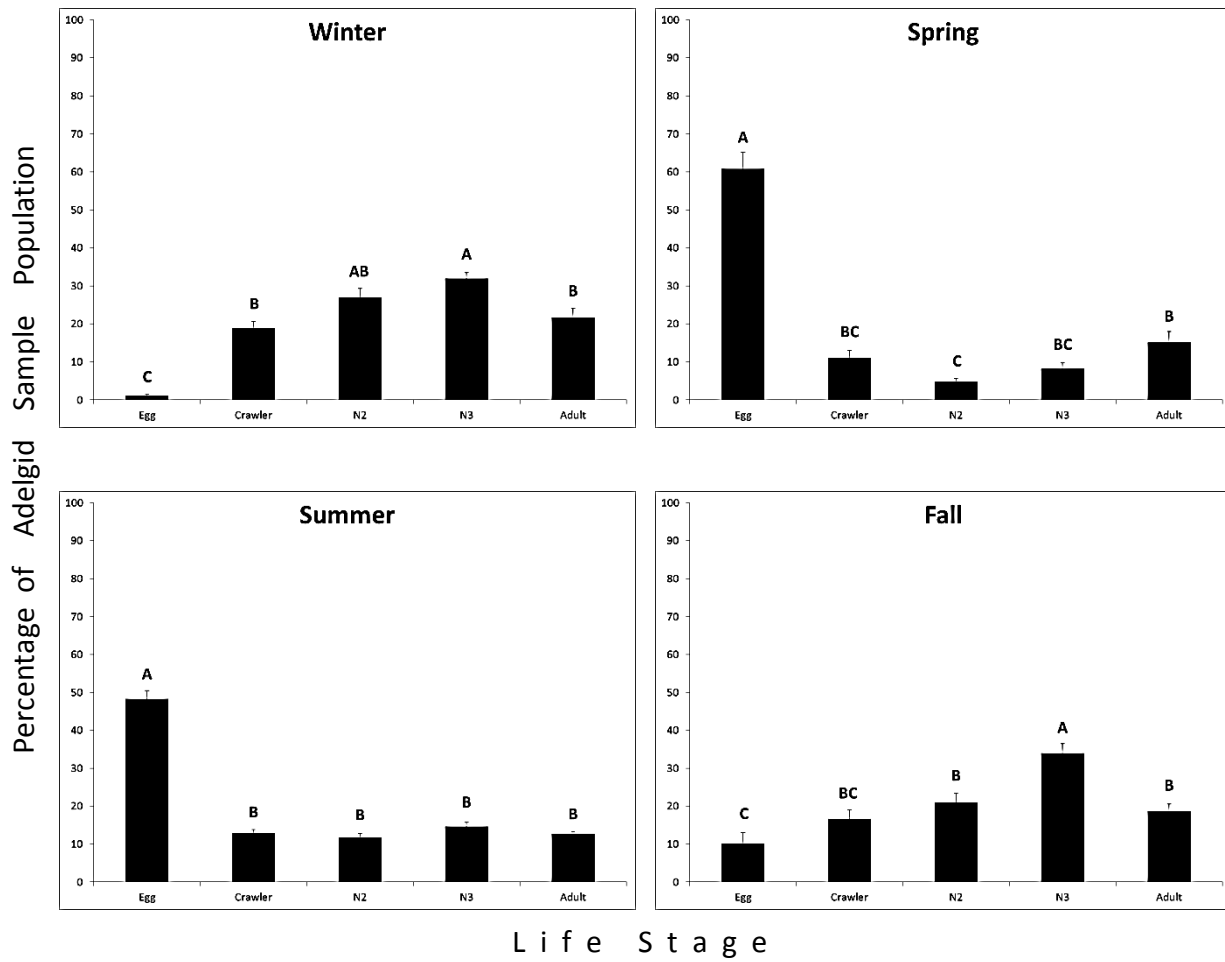


Figure 2.3. Mean percentage (\pm SE) of *P. strobi* life stages within seasons pooled among all sites and years sampled in southwest Virginia in 2014 and 2015. Columns with a letter in common are not significantly different according to analysis using the least square method and Tukey's HSD at $P < 0.05$.

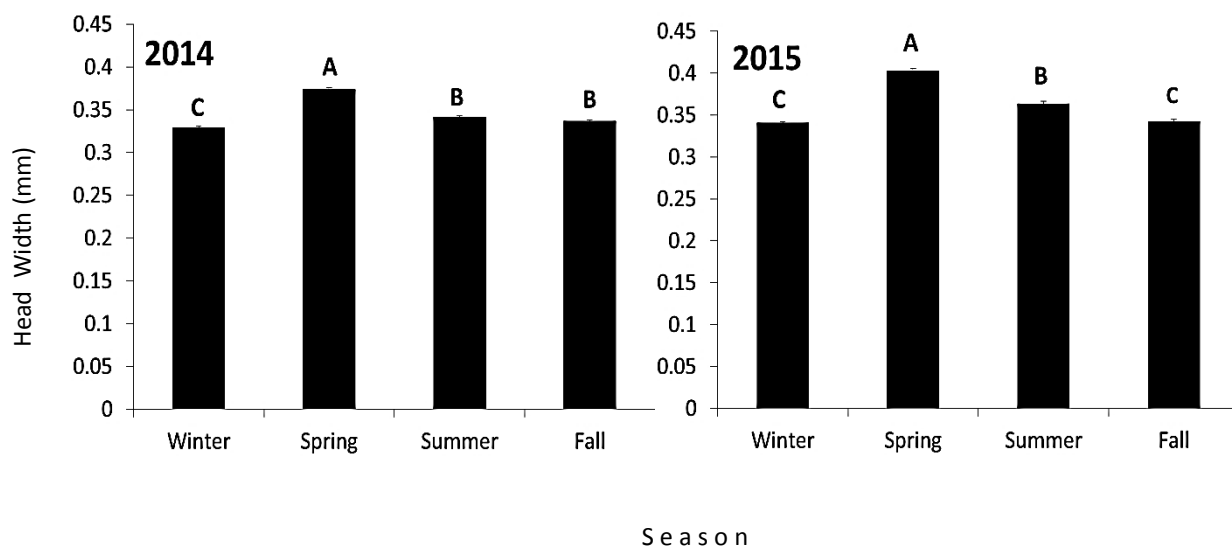


Figure 2.4. Average seasonal head width (mm) \pm standard error of adult *P. strobi* pooled from all sites sampled in southwest Virginia. Columns with a letter in common are not significantly different according to analysis using a one-way ANOVA and Tukey's HSD at $P < 0.05$.

CHAPTER THREE

Predators Associated with the Pine Bark Adelgid, *Pineus strobi*, in Forests of Southwestern Virginia

Abstract

The pine bark adelgid, *Pineus strobi* (Hemiptera: Adelgidae), is native to eastern North America. It is an herbivore specializing on eastern white pine, *Pinus strobus*, with a range stretching from Newfoundland in Canada to the northern tip of Georgia in the US. Little is known about this insect, especially in its southern range, and the composition of its predator complex has not yet been documented in this region. The current study identifies arthropod predators associated with the pine bark adelgid in forests in southwest Virginia based on a two-year survey. Predators were identified using morphology and DNA barcoding. Predator species include: *Laricobius rubidus* (Coleoptera: Derodontidae), a native adelgid specialist, and two species from the family Chamaemyiidae, *Leucopis piniperda* Malloch (Diptera: Chamaemyiidae) and *L. argenticollis* Zetterstedt (Diptera: Chamaemyiidae), that are known adelgid specialists. Also found were predators from the families Cecidomyiidae, Coccinellidae, Chrysopidae, Hemerobiidae, and Syrphidae. The Cecidomyiidae were especially diverse, with 15 different species inferred from their DNA barcodes. Knowledge of this predator complex is valuable in shedding light on the ecology of a little-studied species. It is also useful information for avoidance and detection of potential interactions between native species and those that may be considered for introduction for biological control of other invasive adelgids.

Introduction

The pine bark adelgid, *Pineus strobi* (Hartig) (Hemiptera: Adelgidae), is an herbivore that feeds primarily on eastern white pine, *Pinus strobus* L. (Doane 1961). Although it is an occasional aesthetic pest on Christmas trees and ornamentals, feeding by *P. strobi* does not appear to impact *P. strobus* health, even when adelgid densities are high (Clark and Brown 1960). Thus, little, if any, *P. strobi* management is necessary. The species has been reported in the Pacific northwestern US on *Pinus monticola* (Douglas ex D. Don), and its pest status there is undetermined (Darr 2017). It is also an occasional nursery pest in the UK (Matosevic and Zivkovic 2013)

While *P. strobi* is not considered a significant pest, it shares its range with non-native adelgids that inflict substantial ecologic and economic damage. One invasive species is the hemlock woolly adelgid, *Adelges tsugae*, Annand (Hemiptera: Adelgidae), an ecologically and economically destructive invasive species introduced to the eastern U.S. from Japan (Havill et al. 2016) that feeds on hemlock species (McClure 1991). Another is the balsam woolly adelgid, *Adelges piceae*, Ratzeburg (Hemiptera: Adelgidae). The balsam woolly adelgid from Europe and East Asia is invasive across the U.S. and Canada and causes high mortality in native North American *Abies* spp. (Jenkins 2003, McManamay 2011). Additionally, the pine adelgid, *Pineus boernerii* Annand (Hemiptera: Adelgidae), is introduced in New England where it is reported to damage red pine, *Pinus resinosa* Ait (McClure 1982, CABI 2017). The origin of *P. boernerii* is not clear, but it may be native to East Asia (McClure 1984). Biological control is an important option for controlling invasive adelgids on a landscape scale. It is reasonable to consider that predators of *P. strobi* may also utilize these related alien species because they share habitats.

In the northern reaches of *P. strobi*'s native range, a number of naturally occurring predators have been identified from the families Coccinellidae, Chamaemyiidae, Cecidomyiidae, and Hemerobiidae, as well as *Laricobius rubidus* LeConte (Coleoptera: Derodontidae) (Wilson 1938, Raske and Hodson 1964, Sluss and Foote 1973, Montgomery and Lyon 1995a). *Laricobius rubidus* is considered a specialist predator of *P. strobi*, and is not reported on other prey native to eastern North America (Brown 1944, Clark and Brown 1960, Lawrence and Hlavac 1979, Montgomery and Lyon 1995a). Previous studies of *L. rubidus* phenology report that adults are present from March to June and eggs are laid within *P. strobi* ovisacs during April and May (Clark and Brown 1960, Zilahi-Balogh et al. 2005). There are four instars, each of which, like the adult, feeds on all *P. strobi* life stages. The last instar is believed to drop to the soil for pupation (Clark and Brown 1960). This phenology is highly synchronized with that of *P. strobi*. In southwestern Virginia, *P. strobi* reproduction begins in early April with a large spike in egg production following an apparent quiescent period during the winter (Wantuch et al. 2017).

Following the introduction of the hemlock woolly adelgid, it became apparent that *L. rubidus* could also feed and complete development on this prey species (Montgomery and Lyon 1995b, Zilahi-Balogh et al. 2005, Mausel et al. 2008, Story et al. 2012). *Adelges tsugae* has a similar life cycle to *P. strobi* in that the majority of individuals are wingless, immobile following their first instar, and reproduce parthenogenically (Raske and Hodson 1964, McClure 1989, Havill and Footit 2007). However, there are differences in that *P. strobi* is not active in the winter (Raske and Hodson 1964, Wantuch et al. 2017); whereas *A. tsugae* has two distinct generations per year with nymphs developing to adults by late winter and laying eggs in late winter/early spring, and adult *A. tsugae* aestivate over much of the summer (McClure 1989, Lamb et al. 2007, Mausel et al. 2008). In contrast, *P. strobi* does not diapause and has overlapping generations resulting in all

live stages being apparently available to predators from March-November in southwestern Virginia (Wantuch et al. 2017). Because forest stands of *Pinus strobus* are often intermixed with eastern hemlocks, *Tsuga canadensis* (L.) and Carolina hemlock, *Tsuga caroliniana* Engelman (Burns and Honkala 1990), *L. rubidus* has ample opportunity for interaction with *A. tsugae*. Similarly, *L. nigrinus* Fender (Coleoptera: Derodontidae), a close relative of *L. rubidus* from the Pacific northwestern US has been released in the eastern US as a biological control agent of *A. tsugae* (Mausel et al. 2010, Davis et al. 2011). These two *Laricobius* species are known to produce fertile hybrids in the field, and both species and their hybrids are all known to feed on *A. tsugae* and *P. strobi* (Havill et al. 2012, Fisher et al. 2015).

Little is known regarding predators of *P. strobi*, particularly in the southern portion of its range. Host switching of *Laricobius* species, and possibly other predators, between native and non-native adelgids warrants investigation of the incidence and phenology of *P. strobi* predators in its previously-unstudied southern range. The predator complex of *P. strobi* may exert pressure on these exotic adelgid species, and newly introduced biological controls could impact the native community of predators on *P. strobi*. The current study documents the predator complex associated with *P. strobi* in the forests of southwestern Virginia, with specific attention to the phenology of *L. rubidus*.

Materials and Methods

Specimens were collected from three sites in southwestern VA: Peak Creek, at Gatewood Park and Reservoir, Pulaski, N37.06217° W80.83633°; Hussy Mountain, in Mount Rogers National Recreation Area, Speedwell, N36.77223° W81.17521°; Dismal Falls, in George Washington and

Jefferson National Forest, Giles, N37.19263° W80.89230°. Sampling occurred approximately every two weeks from 13 September, 2013 – 28 April, 2016. This coincided with a phenological study of *P. strobi* at these same sites (Wantuch et al. 2017). Samples were taken from eastern white pine trees colonized by *P. strobi* and were limited to parts of trees accessible from the ground with hand pruners. Mature trees, saplings, and seedlings were sampled opportunistically wherever *P. strobi* was present. Because of the patchy distribution of this insect, it was not possible to implement a structured sampling scheme with regard to tree age class. At each site, on each sampling date, a minimum of 50 trees infested with *P. strobi* were sampled using a beat sheet. Additionally, excised infested branch tips and bark samples were collected during each visit and examined under a dissecting microscope in the laboratory and predators were removed. All predator specimens were stored in a freezer at -20° C, and immature insects were placed in 95% ethanol.

Adult insects were identified using morphological characters; voucher specimens are deposited at the Virginia Tech Insect Museum. All larval specimens were identified using DNA barcoding, with the exception of *L. rubidus*. *Laricobius* larvae can be identified to genus using morphology, but cannot be distinguished to species morphologically, and so it is possible to confuse *L. rubidus* and *L. nigrinus*. The collection sites were located in a region that should only have *L. rubidus*, but a subset of larvae were identified with DNA barcodes (Davis et al. 2011) to ensure that the non-native *L. nigrinus* or their hybrids were not present in the populations. All other predator larvae were identified by comparing DNA barcodes to records in the Barcode of Life Database (BOLD; Ratnasingham and Hebert 2007). For all specimens, DNA was extracted using the Mag-Bind Blood & Tissue Kit (Omega Bio-Tek, Norcross, Georgia). The cuticles were preserved after proteinase digestion and slide mounted as vouchers, which were deposited at the

Yale Peabody Museum of Natural History (YPM). The 5' end of mitochondrial cytochrome c oxidase subunit I (COI) was amplified using primers LepF1 and LepR1 (Herbert et al. 2004), and sequencing was performed at the DNA Analysis Facility on Science Hill, Yale University using an ABI 3730 sequencer (Life Technologies, Grand Island, NY). Chromatograms were edited using Geneious v7 (<http://www.geneious.com>, Kearse et al. 2012). Samples were identified to the most specific taxon possible by searching the BOLD database (www.barcodinglife.org).

Results

Laricobius rubidus

A total of 156 adult *L. rubidus*, 44 larvae, and 17 eggs were collected at all three sites over the course of the study (**Table 3.1**). Adult *L. rubidus* were consistently active beginning in the fall during October of 2013 and early December of 2014 and 2015 (**Figure 3.1**). Across all years there was a period of inactivity during the coldest parts of the winter in which no adults were collected. In 2013, this occurred from December to late February. In 2014 no beetles were observed from January to mid-February, and in 2015, adults were not collected from January to April. Additionally, 2 eggs were found in late March of 2013, one egg was found in March of 2015, 11 eggs and 9 larvae recovered from branch tips in April, 2015, 3 eggs were found in March of 2016, and 33 larvae were collected from branch tips in April of 2016. *Laricobius rubidus* activity ceased no later than May and appeared to have only one generation per year.

Coccinellidae

Adult lady beetles (Coleoptera: Coccinellidae) were collected October of 2013, May to October of 2014, and April to late September of 2015. Larvae were observed from May to July of 2014

and 2015 (**Table 3.1, Figure 3.2**). Specimens identified using morphological characters include: *Harmonia axyridis* Pallas, *Scymnus indianensis* Weise, *Scymnus* spp. of the subgenus *Pullus*, and *Brachiacantha ursina* Fabr..

Chamaemyiidae

Leucopis argenticollis Zetterstedt (Diptera: Chamaemyiidae) larvae were collected from branch tips from April – August and *L. piniperda* Malloch from June to July. 5 empty *Leucopis* puparia were collected from mid-February through late March and in September of 2014, but it was not possible to identify these to species. One puparium was collected in December of 2015 from which an adult *L. argenticollis* emerged (**Table 3.1, Figure 3.3**).

Cecidomyiidae

Cecidomyiid (Diptera: Cecidomyiidae) larvae were found on branch tips from April to October (**Table 3.1, Figure 3.4**). Fifteen putative species were identified using DNA barcoding, however none matched other records identified to species. Specimens from eight of these putative species (Species 3, 5, 6, 7, 12, 14, and 15) were collected from within *P. strobi* ovisacs, suggesting that they are likely predaceous. An individual of Species 6 was observed eating an adult adelgid and its eggs. Specimens of the other putative species were collected on or near the adelgids.

Neuroptera

Hemerobiid larvae were observed in the field during late April and May of 2014 and 2015 and an adult in December of 2013 (**Table 3.1**). Larval DNA barcoding was inconclusive; sequences recovered were matched to those of known hemerobiid prey, suggesting that sequences

originated either from larval gut contents or from remnants of prey carcasses that they are known to carry as camouflage. Three *Glenochrysa lineaticornis* Fitch (Neuroptera: Chrysopidae) were collected on 18 September, 2015 (**Table 3.1**).

Syrphidae

Four species of syrphid larvae (Diptera: Syrphidae) were collected: *Allograpta obliqua* Say (Diptera: Syrphidae) in May of 2015, *Syrphus torvus* Osten Sacken (Diptera: Syrphidae) in April, 2016, *Toxomerus geminatus* Say (Diptera: Syrphidae) in June of 2014 and October of 2015, and *Eupeodes* sp. in September of 2013 (**Table 3.1, Figure 3.3**).

Discussion

The phenology of *L. rubidus* observed in this study differs markedly from previous observations in the beetle's northern range where adults were found from March to June and eggs were laid in April and May (Clark and Brown 1960, Zilahi-Balogh et al. 2005). The present study finds *L. rubidus* present as early as mid-October through the beginning of May. It is apparently absent for a period during the winter from late December to mid February. Eggs were found March to April, and larvae were collected in April (**Figure 3.1**). There appears, therefore, to be an approximately one month shift in activity between southern and northern ranges of *L. rubidus* from late winter through summer with regard to adult, egg, and larval presence, which may be explained by warmer southern temperatures. Like *L. nigrinus*, *L. osakensis* and *L. erichsonii* Rosen (Coleoptera: Derodontidae), the European predator of *A. piceae*, *L. rubidus* was observed to be univoltine (Buffama 1962, Vieira et al. 2013, Zilahi-Baloh et al. 2003). More striking is *L. rubidus* activity during the late fall and early winter. During these months, all life stages of *P.*

strobi are present, but eggs are scarce and most adults are not actively reproducing (Wantuch et al. 2017). This behavior is synchronous with that of *A. tsugae*, which is most active from late winter through early spring (McClure 1989, Mausel et al. 2008). *Laricobius rubidus* is known to complete development on *A. tsugae* in the laboratory, and it is not uncommon to find it feeding on *A. tsugae* in the field (Montgomery and Lyon 1995b, Zilahi-Balogh et al. 2005, Mausel et al. 2008, Havill et al. 2012, Story et al. 2012, Fischer et al. 2015). It appears that this native predator, which specialized on *P. strobi*, has adapted to take advantage of this abundant invasive prey source.

Two species of chamaemyiid flies were found on branch tip samples infested with *P. strobi*. The predaceous larval stage was observed from May to August in 2014 and in April of 2016 (**Figure 3.3**). This finding is significant because both *L. argenticollis* and *L. piniperda* are known to be predators of *A. tsugae* on the west coast of the US, where that adelgid is native. They are currently being released as biological control agents for *A. tsugae* (Ross et al. 2010, Grubin et al. 2011, Kohler et al. 2016, Motley et al. 2017). The current study shows that both species are present in the environment prior to release, which may aid establishment of the released strains and ease ecological concerns regarding introduction of foreign organisms to a new ecosystem. Not represented in the survey of predators was *Neoleucopis pinicola* Malloch (Diptera: Chamaemyiidae), a species previously found feeding on *P. strobi* (Clark and Brown 1957).

A large number of cecidomyiid gall midge larvae were found on branch tips in association with *P. strobi*. They were collected beginning in early April of 2014 and 2015 through September of 2014 and October of 2015; they were also collected in September of 2013 (**Figure 3.3**). DNA barcoding results suggest that there are 15 distinct species represented, although none match records that have been identified to species. This is not surprising; little systematic work has

been done on this group, and it promises to be highly diverse (Gagné 1989, Mamaev and Krivosheina 1965). In fact, Herbert et al. (2016) suggested that it is likely the most diverse insect family in all of Canada after an extensive survey of over one million samples using DNA barcodes. Cecidomyiids were also a large portion of predators collected by Wallace and Hain (2000) in western North Carolina in association with *A. tsugae*, though they were only found in June and July. Cecidomyiids have a broad range of life histories. Most are plant feeders, but some feed on fungi, sap, or are predators (Gagné 1989, Mamaev and Krivosheina 1965). Although it is possible some species collected in this study are not predaceous and were perhaps feeding on tree sap or fungi, one species was directly observed consuming *P. strobi*, and the presence of larvae from 7 other species found within adelgid ovisacs is certainly suggestive of predatory behavior.

The presence of several generalist predators is consistent with other studies of adelgid predator communities (Montgomery and Lyons 1995ab, Wallace and Hain 2000). Adult and larval coccinellids were commonly collected from April to October; among them were *H. axyridis*, *Brachiacantha ursina*, *Scymnus indianensis*, and other *Scymnus* spp. from the subgenus *Pullus* (**Figure 3.2**). Several members of this family are predators of other adelgids in other regions. Wallace and Hain (2000) found *H. axyridis* to be very common on *A. tsugae* in western NC as well, but only briefly, from late March – June whereas the current study found them in large numbers later in the year during October. Green and brown lacewing larvae as well as syrphid larvae were also found in association with *P. strobi*. It is important to note, however, that it is not possible to say for certain that these predators were feeding on *P. strobi*.

This study is the first to document the predator complex associated with *P. strobi* in its southern range. Several significant findings were made. The native *P. strobi* specialist *L. rubidus* has an expanded period of activity, which may allow it greater opportunity for interaction with the invasive *A. tsugae*, as well as the closely related biological control agent *L. nigrinus* with which it hybridizes. *Laricobius rubidus* has been observed mating with *L. nigrinus* in the field (Mausel et al. 2008), and the pairings are known to produce fertile offspring (Havill et al. 2012). The result is a geographic mosaic of hybridization between the two *Laricobius* species (Fischer et al. 2015). Despite concern regarding threat to *L. rubidus* species integrity and resulting ecological repercussions, hybridization may have aided in establishment of *L. nigrinus* to the eastern US (Havill et al. 2012). Recent studies indicate that complete hybrid assimilation is unlikely due to the strong respective host preferences of each species (Fischer et al. 2015). Additionally, two species of chamaemyiid silver flies currently under consideration as *A. tsugae* biocontrol agents were found to be present in the environment already and apparently feeding and developing on *P. strobi*. Many cecidomyiid gall midges were collected, some of which are likely adelgid predators. Species 3 and species 5 with 33 and 11 specimens, respectively, were the most abundant and are therefore most likely to have the strongest impact on *P. strobi* populations and could be investigated further. Although the cecidomyiid community on *P. strobi* remains to be fully characterized, this study provides valuable data in advance of their official identification. It is notable that the *P. strobi* predator complex includes members of the derodontid and chamaemyiid families, both of which specialize on adelgids. This is consistent with other described adelgid predator guilds, as is the presence of cecidomyiid midges and coccinellids (Humble 1994, Kohler 2008). Not only does this work describe the network of species that may utilize the native adelgid, *P. strobi*, it may also serve to inform future biological control

programs designed to manage invasive adelgid species with which it shares its environment. Additionally, increased knowledge of the predator-prey interactions involving *P. strobi* will make any disturbance to the native ecosystem by introduced predators, such as those intended for *A. tsugae* management, easier to both detect and avoid.

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Table 3.1. Predatory species found associated with *Pineus strobi* on *Pinus strobus* in southwestern Virginia with number of each life stage collected (eggs, larvae, pupae, adults).

Specimens identified to various levels; counts of broader levels include those of narrower levels within them. Cecidomyiid specimens could not be identified to species; those that could be grouped together based on their DNA barcode index numbers (BINs) are given arbitrary names and their BINs are shown in parentheses.

Order	Family	Genus	Species
Coleoptera (1,61,0,241)	Derodontidae (17,44,0,156)	<i>Laricobius</i> (17,44,0,156)	<i>rubidus</i> (17,44,0,156)
	Coccinellidae (0,17,0,85)	<i>Harmonia</i> (0,0,0,65)	<i>axyridis</i> (0,0,0,65)
		<i>Scymnus</i> (0,0,0,15)	<i>indianensis</i> (0,0,0,1)
		<i>Brachiacantha</i> (0,0,0,1)	<i>ursina</i> (0,0,0,1)
		<i>Diomus</i> (0,0,0,1)	<i>terminatus</i> (0,0,0,1)
Diptera (2,107,7,0)	Chamaemyiidae (1,24,7,0)	<i>Leucopis</i> (1,22,1,0)	<i>argenticollis</i> (1,20,1,0)
			<i>piniperda</i> (0,2,0,0)
	Cecidomyiidae (0,74,0,0)	----	Species 1 (BOLD:AAD8276) (0,1,0,0)
			Species 2 (BOLD:AAB2384) (0,1,0,0)
			Species 3 (BOLD:AAQ0655) (0,33,0,0)
			Species 4 (BOLD:ACK9896) (0,1,0,0)
			Species 5 (BOLD:AAH3755) (0,11,0,0)
			Species 6 (BOLD:AAY6419) (0,2,0,0)

			Species 7 (BOLD:ACK3142) (0,6,0,0)
			Species 8 (BOLD:AAY6411) (0,2,0,0)
			Species 9 (BOLD:ACL3715) (0,1,0,0)
			Species 10 (BOLD:AAV5558) (0,2,0,0)
			Species 11 (BOLD:ABW7837) (0,3,0,0)
			Species 12 (BOLD:AAH3720) (0,3,0,0)
			Species 13 (BOLD:AAM6059) (0,1,0,0)
			Species 14 (BOLD:AAH3734) (0,1,0,0)
			Species 15 (BOLD:ACC8281) (0,1,0,0)
	Syrphidae (0,6,0,0)	<i>Toxomerus</i> (0,2,0,0)	<i>geminatus</i> (0,2,0,0)
		<i>Syrphus</i> (0,1,0,0)	<i>torvus</i> (0,1,0,0)
		<i>Allograpta</i> (0,2,0,0)	<i>obliqua</i> (0,2,0,0)
		<i>Eupeodes</i> (0,1,0,0)	----
Neuroptera (0,18,0,1)	Hemerobiidae (0,3,0,1)	----	----
	Chrysopidae (0,3,0,0)	<i>Glenochrysa</i> (0,3,0,0)	<i>lineaticornis</i> (0,3,0,0)

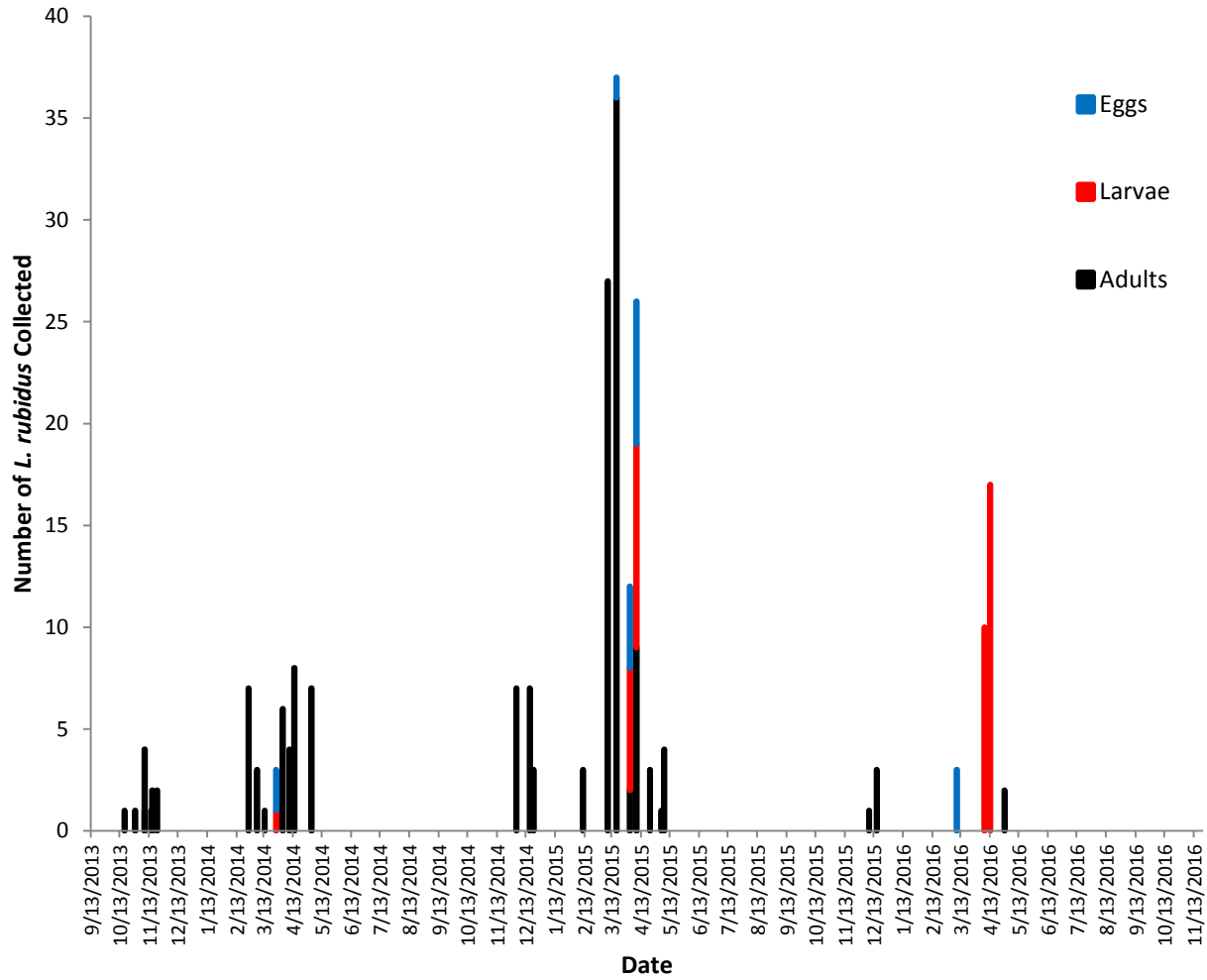


Figure 3.1. Number of *L. rubidus* of each life stage collected on each sampling date between fall 2013 and fall 2016 in southwest Virginia.

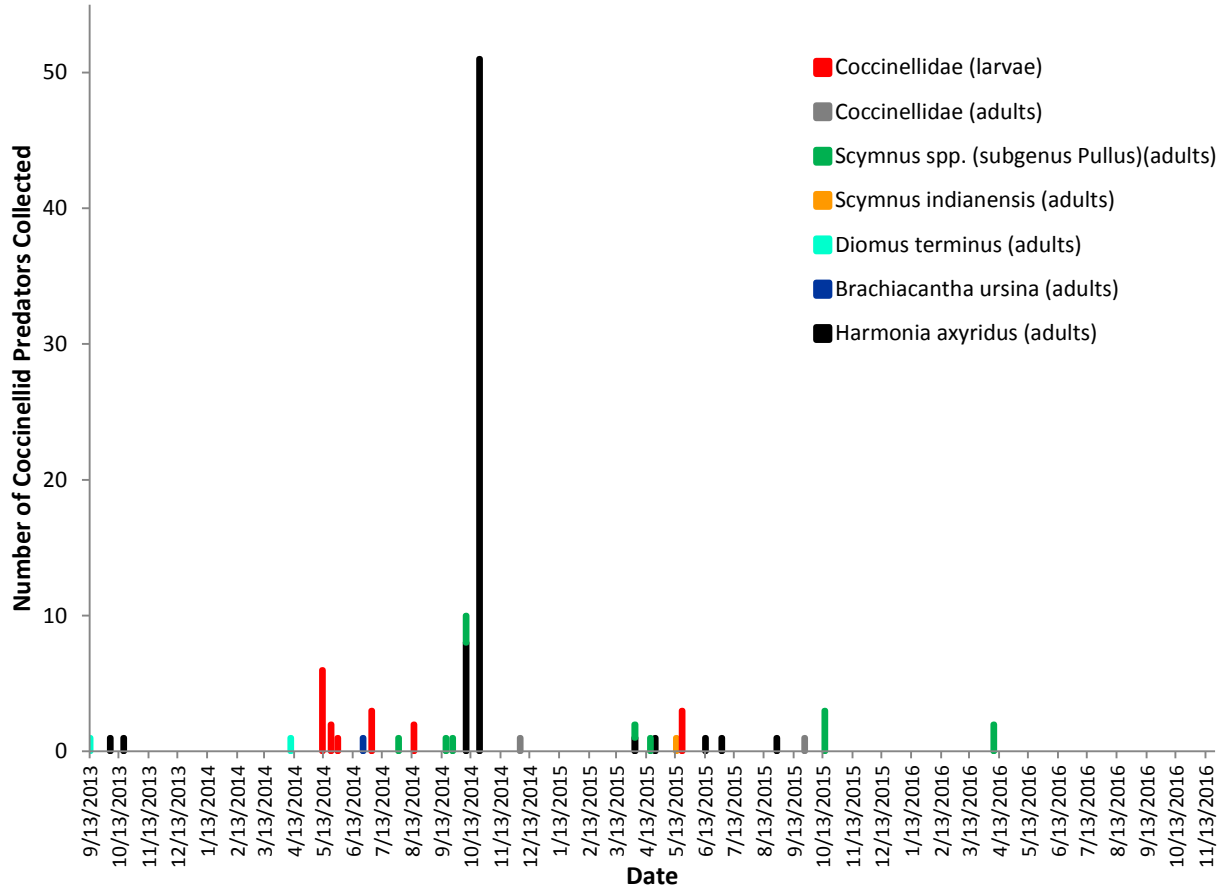


Figure 3.2. Number of coccinellid predators associated with *P. strobi* collected on each sampling date between fall 2013 and fall 2016 in southwest Virginia. Specimens not identified beyond family are shown as Coccinellidae (larvae) and Coccinellidae (adults), respectively.

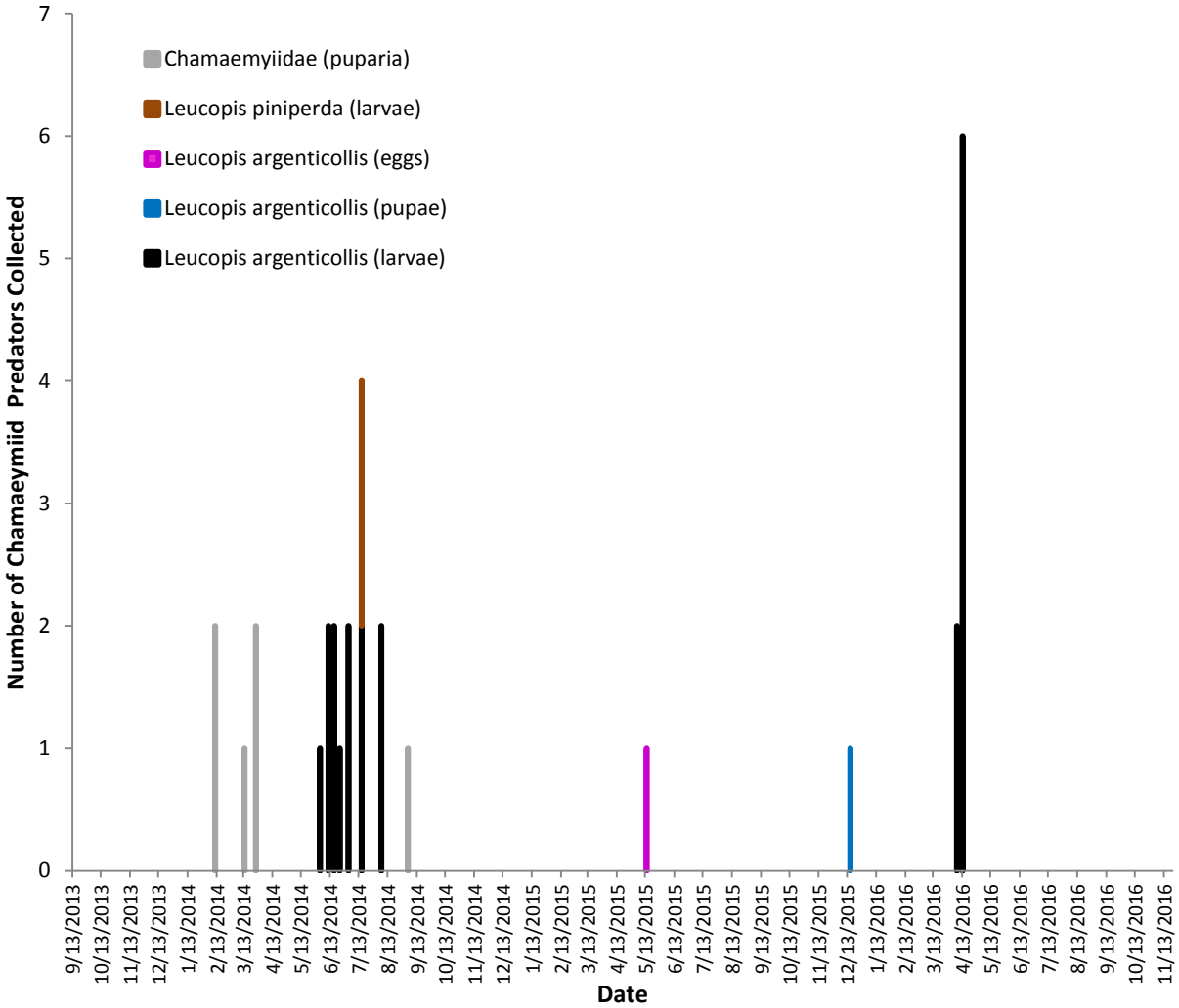


Figure 3.3. Number of chamaemyiid predators associated with *P. strobi* collected on each sampling date between fall 2013 and fall 2016 in southwest Virginia. Specimens not identified beyond family are shown as Chamaemyiidae (puparia).

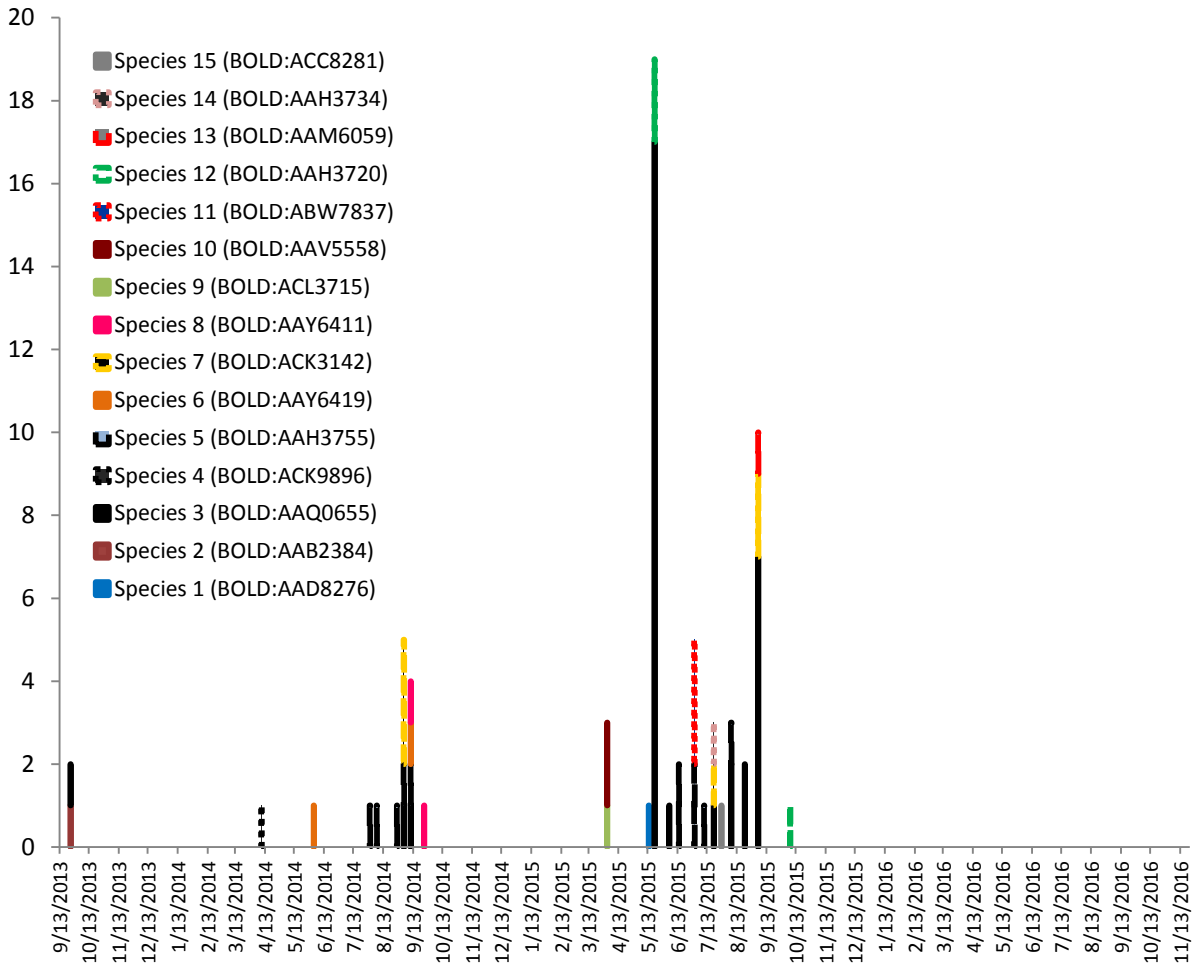


Figure 3.4. Number of cecidomyiid predators associated with *P. strobi* collected on each sampling date between fall 2013 and fall 2016 in southwest Virginia. Specimens could not be identified by species but were grouped by barcode index number (BIN) and given arbitrary species designations.

CHAPTER FOUR

Supercooling Points of *Laricobius rubidus* in Southwestern Virginia

Abstract

Laricobius rubidus LeConte (Coleoptera: Derodontidae) is a predator of the pine bark adelgid, *Pineus strobi* Hartig (Hemiptera: Adelgidae) native to eastern North America. Two related species of adelgid predators, *L. nigrinus* Fender and *L. osakensis* Montgomery and Shiyake, have been released in the same range as biological control agents of the hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae). Susceptibility of *L. rubidus* and *L. osakensis* to cold temperatures has not been investigated. The present study quantifies the super-cooling point of *L. rubidus*, an indicator of relative cold-hardiness, from November – March, 2016. These results are intended for comparison to those of a parallel study of *L. nigrinus* and *L. osakensis*.

Introduction

Laricobius rubidus is a native specialist predator of the pine bark adelgid, *Pineus strobi* (Hartig) (Hemiptera: Adelgidae) (Clark and Brown 1960, Chapter 2). *Pineus strobi* is an herbivore specialist of eastern white pine, *Pinus strobus* L. (Doane 1961). As such, both *P. strobi* and *L. rubidus* are distributed throughout the range of *P. strobus* (Annand 1928, Brown 1944).

Although *L. rubidus* is the only species of its genus native to the eastern U.S., two congeners, *L. nigrinus* and *L. osakensis*, have been introduced into this region as biological control agents of the hemlock woolly adelgid, *Adelges tsugae* (Mausel et al. 2010, Mooneyham et al. 2016).

Laricobius nigrinus is native to the western temperate forests of North America (Mausel et al. 2017) and *L. osakensis* to Japan (Montgomery et al. 2011).

Because *P. strobi* appears to experience negligible winter mortality (Wantuch et al. 2017), while *A. tsugae* shows much higher susceptibility to low temperatures (McAvoy et al. 2017, Tobin et al. 2017), it is possible that their respective predators may also express varying degrees of cold hardiness. Synthesis of cryoprotectants is one strategy by which insects cope with sub-freezing temperatures (Salt 1961, Sinclair 2003). Thus, the supercooling point, the temperature at which body fluids crystalize, is a quantifiable indicator of relative cold hardiness. The following study investigates the supercooling points of *L. rubidus* collected in southwestern Virginia from November 2016 – March 2017. It is intended to complement a parallel study measuring the supercooling points of *L. osakensis* (northern and southern strains) and *L. nigrinus* exposed to the same climactic conditions during the same time period.

Materials and Methods

Laricobius rubidus adults were collected from Dismal Falls, in George Washington and Jefferson National Forest, Giles, VA, N37.19263° W80.89230°, which lies in plant hardiness zone 6b (USDA Plant Hardiness Zone Map 2012). Beetles were collected from *P. strobi* – infested *P. strobus* trees using a beat sheet. Samples were collected in early and mid-November, 2016, mid-December, 2016, late January, 2017, and mid-March, 2017. Inclement weather prohibited sampling in February.

Following collection, specimens were kept at 0°C for 24 hr without access to food or water. Equipment and methods used to measure supercooling points were the same as described in

Bentz and Mullins (1999) with the following exceptions: the circulator bath used was Lauda K-2/R (Lauda-Brinkmann, Delran, NJ), data were acquired using DaqTemp (IOtech Inc., Cleveland, OH), and software used was Daqview v.7.13.14 (Iotech Inc., Cleveland, OH). Beetles were placed on a template in contact with a thermocouple using a drop of zinc oxide thermal grease. Specimens were placed on a stir-kool plate and cooled at a rate of approximately 3°C/min until freezing occurred. Body temperature of the beetle was measured using the thermocouple, and the supercooling point was interpreted to be the lowest temperature recorded prior to the spike resulting from the release of latent heat of fusion that occurs when fluids within the insect body crystalize due to freezing. Supercooling points were measured from 20 beetles each month with the exception of December when, due to equipment failure, only 5 supercooling points could be attained.

Data were analyzed with JMP Pro 11.0 (SAS Institute, Inc. 2013). A one-way ANOVA was used to test for significant differences in mean supercooling points between months. Means were compared using Tukey's HSD at $P < 0.05$.

Results

The supercooling point of *L. rubidus* averaged between -14 to -17°C during the winter months with the lowest recordings occurring during January and February (**Table 4.1**). Although there was no significant difference in mean supercooling point among the four months of the study period, there was a trend toward lower mean supercooling points for the colder months.

Discussion

Despite a lack of significant differences in mean supercooling point between *L. rubidus* collected monthly during the study, there was a numerical decrease as the months got colder. Temperature trends indicative of what is typical of the region, albeit for previous years, are shown in Wantuch et al. (2017). Mean supercooling point of *L. rubidus* in January, 2017 ($-17.72\text{ }^{\circ}\text{C} \pm 1.40\text{ }^{\circ}\text{C}$) was significantly lower than that of both the northern and southern strains of *L. osakensis* ($-13.1\text{ }^{\circ}\text{C} \pm 0.98\text{ }^{\circ}\text{C}$ and $-13.49\text{ }^{\circ}\text{C} \pm 0.98\text{ }^{\circ}\text{C}$, respectively) in a parallel study (A. Toland and S. Salom, Virginia Tech, *unpublished data*) conducted with intent for comparison to the present one. *Laricobius rubidus* also had significantly lower mean supercooling points in March, 2017 ($-18.1880\text{ }^{\circ}\text{C} \pm 1.2126612\text{ }^{\circ}\text{C}$) than did *L. nigrinus* and *L. osakensis* (both northern and southern strains) measured in February, 2017 (-11.19 ± 1.50 , $-12.40\text{ }^{\circ}\text{C} \pm 1.21\text{ }^{\circ}\text{C}$ and $-10.96^{\circ}\text{C} \pm 1.21^{\circ}\text{C}$, respectively). Collection of *L. rubidus* from field sites in February of 2017 was prevented by inclement weather.

While supercooling point alone cannot elucidate an insect's cold hardiness, it is one aspect of it (Salt 1961). When directly compared between closely related species exposed to the same climactic conditions, supercooling point may prove to be a highly quantifiable indicator of relative susceptibility to low temperatures (Sinclair et al. 2003). Significantly lower mean supercooling points of *L. rubidus* than those of northern and southern strains of *L. osakensis*, as well as those of *L. nigrinus* in January and February/March of 2017 may be suggestive of their comparative cold-hardiness.

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Table 4.1. Mean supercooling points and standard error of the means of *L. rubidus* collected monthly. Error bars indicate standard error of the mean. There were no significant differences among means.

Month	Mean Supercooling Point (°C)	Std Error
November	-13.85	1.37
December	-14.41	3.13
January	-17.72	1.40
March	-18.19	1.40

CHAPTER FIVE

Conclusions

This research examined the ecology of the pine bark adelgid, *Pineus strobi* (Hartig) (Hemiptera: Adelgidae), a native forest insect in southwestern Virginia. Prior to this work, little was known regarding *P. strobi* in its southern range, despite the fact that it exists interspersed with the invasive hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae). This study investigated the phenology and predator complex of *P. strobi*, with additional focus on *Laricobius rubidus* LeConte (Coleoptera: Derodontidae), a native specialist of *P. strobi*, and its cold-hardiness.

I found the seasonal phenology of *P. strobi* to differ from what had been previously described in its northern range. It did not seem to undergo the quiescent period that it did in the colder northern climate. Rather than the majority of the population overwintering as third instars, life stage frequencies were more evenly distributed with sporadic egg production during warm spells. This resulted in greater overlap of generations throughout the rest of the year. These findings are valuable in application to the biological control program of the *A. tsugae*, since *P. strobi* can, in many instances, serve as an alternate food source should *A. tsugae* be scarce due to high winter mortality or in the case of early predator emergence in rearing labs in the fall. This phenological plasticity also shows that, in the event of new introductions of this or related species to a novel range, previously described population dynamics may not necessarily reflect how the species will actually behave. Thus, future introductions or significant climate change may warrant consideration of control measures not currently needed.

When surveying predators associated with *P. strobi*, I found abundant *L. rubidus* with an active period beginning earlier than previously described. In the current study, adult *L. rubidus* were collected from February through May as well as from October through December. They were seemingly absent only during the summer, when they are believed to pupate in the soil, and in the coldest part of the winter. This extended activity allows for greater interaction with *A. tsugae*, a novel invasive prey source, as well as *L. nigrinus* Fender (Coleoptera: Derodontidae), an introduced predator intended for use as a biological control agent. We also identified two other adelgid specialists, *Leucopis piniperda* Malloch (Diptera: Chamaemyiidae) and *Leucopis argenticollis* Zetterstedt (Diptera: Chamaemyiidae). Populations of both of these species collected in the western U.S., where *A. tsugae* is native, are now being released in eastern North America for biological control. Although these strains are genetically distinct, their observation in southwestern Virginia bodes well for their successful establishment. A multitude of cecidomyiid gall midges were found, and although they could not be identified as known species, it was evident from DNA barcoding results that 15 species were represented, at least 8 of which show evidence of predatory habits. A variety of coccinellids was also found in this study, as well as several syrphid, chrysopid, and hemerobiid species. The general assemblage of *P. strobi* shows remarkable similarity in composition to that of other adelgid species in their native ranges. There seems to be consistent association of adelgids with derodontid and chamaemyiid predators with variable prey specificity. Also found in similar surveys of adelgid predators are cecidomyiid midges, coccinellids, chrysopids, and hemerobiids. These similarities across continents seems quite noteworthy.

All manner of future adelgid work would benefit from the construction of a degree day model for adelgid development. Additionally, increased study of the systematics of the family

Cecidomyiidae could potentially reveal an underappreciated adelgid predator. Finally, more extensive studies of the susceptibility to cold of *L. rubidus* focusing on lower lethal limits would be very informative, especially if compared to the cold-hardiness of other introduced *Laricobius* species.