N O T E

A Simple Method of Detecting Hemlock Woolly Adelgid (Hemiptera: Adelgidae) Predator Activity Using Ultraviolet-A Light[1]

R.C. McDonald[2] and L.T. Kok[3]

Symbiont Biological Pest Management, 194 Shull's Hollar Rd. Sugar Grove, North Carolina USA 29679
[3]Department of Entomology, 216 Price Hall, Virginia Tech, Blacksburg VA 24061

J. Entomol. Sci. 49(2): 200-205 (April 2014)

Key Words hemlock woolly adelgid, *Adelges tsugae*, biological control, *Laricobius nigrinus*, *Sasajiscymnus tsugae*

On the evening of 11 March 2011, RCM first observed the fluorescent orange properties of the adelgid predator *L. nigrinus* frass on hemlock woolly adelgid-infested hemlocks at his residence in Sugar Grove, NC. This was done by using an LED UV 'spy pen' (Item #: 11,129 purchased at Archie McPhee's store in Seattle, WA) and shining the UV light on the underside of hemlock branches. The LED UV pen was originally purchased to look for bedbug and other unwanted activity in hotel rooms.

We then hypothesized that UV light of the correct wavelength (A) may be used as a noninvasive technique to view the adelgid's current state and predator activity without having to physically destroy the hemlock woolly adelgid ovisac (egg sack). In the case of 'disturbed' ovisacs, viewing with UV light will produce unique colors indicative of predator activity on an adelgid or its eggs. The presence or absence of these characteristic colors allows us to positively determine adelgid predator activity for each particular 'disturbed' ovisac. In the case of *L. nigrinus*, it is the only predator active during the winter months on hemlock woolly adelgid (Zilahi-Balogh, 2003, Can. Entomol. 135:103 - 115).

A Japanese strain of the hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Sternorrhyncha: Adelgidae) was accidentally introduced into the Richmond, VA area of the eastern USA in the early 1950s on weeping hemlocks from the main island (Honshu) of northern Japan (*Tsuga diversifola* (Maxim.) Masters 1881). The adelgid was originally thought to be native only to Asia; however, in 2006, the hemlock woolly adelgid was found by the US Forest Service to be native to the Pacific Northwest of the USA and Canada on western (*T. heterophylla* (Rafinesque) Sargent 1898) and mountain (*T. mertesiana* Sarg.) hemlocks (Havill et al. 2006, Ann. Entomol. Soc. Am. 99(2):195 - 203). The hemlock woolly adelgid is not considered to be a pest

[1]Received 24 July 2013; accepted for publication 19 September 2013.
[2]Corresponding author (email: drmcbug@skybest.com).

in the western US where it is native (Furniss and Carolin 1977, USDA FS Misc. Pub. # 1339). Hemlock woolly adelgid populations have subsequently spread to most of the eastern U.S. with hemlocks and are currently causing significant mortality of eastern (*Tsuga canadensis* (L.) Carrière)) and Carolina (*Tsuga carolinensis* Engelmann) hemlocks throughout most of their range (McClure 1989, Ann. Entomol. Soc. Am. 82:50; http://na.fs.fed.us/fhp/hemlock woolly adelgid/maps/2012.pdf).

The hemlock woolly adelgid has 2 generations per year; a sistens generation that lasts from midOctober until May, and a short progrediens generation that lasts from May until June (McClure 1989). Settled sistens stage adelgids aestivate over the warmer summer months at the base of needles on new hemlock growth until the onset of cooler temperatures by midOctober. At this time, the sistens break aestivation, begin feeding, and produce a spherical, waxy, wool-like substance (egg sac or ovisac) covering their body. The ovisac provides a measure of protection from the environment, and may also protect the adelgid from some generalist predators. Adelgid honeydew is passed through the ovisac and rests on the outside of it as a droplet, which increases in size as the adelgid grows to maturity.

Researchers found that natural enemies, particularly predatory beetles, are critical in regulating populations of hemlock woolly adelgid below damaging levels in both the Pacific Northwest (Zilahi-Balogh 2003, Kohler et al. 2008, Environ. Entomol. 37: 494 - 504) and Asia (McClure and Cheah 1999, Biol. Inv. 1: 247 - 254; Montgomery et al. 2000, USDA FS Gen. Tech. Rep. NE-276; Yu et al. 2000, The Col. Bull. 54:154 - 199). Studies in the Pacific Northwest showed the native predatory beetle *Laricobius nigrinus* Fender (Coleoptera: Derodontidae) is a significant natural enemy of the hemlock woolly adelgid there. *Laricobius nigrinus* is unique in that it emerges during the fall, and is active all winter, when all other adelgid predators are absent. The *L. nigrinus* adults and their larvae feed on the hemlock woolly adelgid sistens adults, progrediens eggs, and progrediens crawlers until late April or early May; when the *L. nigrinus* larvae drop to the needle duff under the tree and pupate. Thus, they attack and hold in check the sistens generation, which is the largest and most damaging of the 2 adelgid generations each year (Mausel et al. 2008, Environ. Entomol. 37: 1498 - 1507).

In 2003, researchers at Virginia Tech, with support from the US Forest Service, reared, released, and established *L. nigrinus* populations (by 2006) in northwestern North Carolina (McDonald et al. 2011, Ch. 16. USDA FS Pub. FHTET-2011 - 04). Positive *L. nigrinus* populations also have been established and reported from Georgia, MD, NJ, Tennessee, VA, and West Virginia (Mausel et al. 2011, Ch. 6 USDA FS Pub. FHTET-2011 - 04). Researchers during the 1990s also imported, released and established *Sasajiscymnus tsugae* (Coleoptera: Coccinellidae), a Japanese predator of the hemlock woolly adelgid progrediens generation active during the later spring and summer months (Cheah and McClure, 2000, Agric. For. Entomol. 2:241 - 251).

Determining accurate predation rates of the hemlock woolly adelgid sistens generation entails clipping infested hemlock twigs, (usually in late March or early April; sistens adelgid peak egg laying phenology is synced with redbud tree bloom), and returning these clipped twigs to the laboratory. The adelgid ovisacs are then dissected under a 30X or greater power dissecting scope. Predation rate estimates occur during the 2 peak egg-laying periods of the hemlock woolly adelgid – March and April for the sistens generation attacked by *L. nigrinus*, and late May and June for the progrediens generation attacked by *S. tsugae*. These 2 peak egg-laying periods are also when we have seen that peak fluorescent colors can be observed using UV-A light.

Ovisac dissections under a dissecting scope can determine the presence of predator eggs, predator larvae, and their physical evidence (predator frass and physically damaged ovisacs) relative to the number of living adult adelgids, adelgid eggs, and crawlers. This allows researchers to determine percentage rates of predation by *L. nigrinus* and other predators. Originally, adelgid ovisacs that were physically damaged but had no predators present were classified separately from ovisacs with predators. We could not, at that time, accurately determine if these 'disturbed' ovisacs were actually caused by predatory activity; since we were unable to directly observe predators feeding on adelgid prey under the scope.

The dissection of adelgid ovisacs is a time-consuming, tedious process that must be performed under a 30X dissecting scope or similar magnifying device, such as a knitting loupe, for accuracy. Ovisacs were classified as positive for predation if a *L. nigrinus* (the sistens generation ovisac) or *S. tsugae* (the progrediens generation ovisac) predator egg or larva was found inside. Ovisacs were classified as 'disturbed' if the mechanical and predatory actions of predators had torn open and disrupted the ovisac, without the predator being physically present at the time of observation.

During March and April, hemlock woolly adelgid-infested twigs were excised from hemlock trees and transported to the laboratory, where they were placed into a darkened room in a laboratory or a photography darkroom. Using a standard commercially available 15-W 'black light poster' UV-A fluorescing tube, the hemlock twigs were placed directly under the UV light to detect the presence of colors indicating predator activity. We also examined adelgids in an area known to have established populations of *S. tsugae* during May. Our experience since 2011 showed us that fluorescent UVA light gave richer colors and was more illuminating than LED UV light. The more powerful the UV wattage, the brighter were the corresponding reflected colors and eye protection must be considered.

Starting in March of 2011, we also began using portable, hand held UV-A fluorescent 'pet urine detector' lights in the 400 - 315 nm wavelength at night in the field to illuminate adelgid-infested hemlocks known to have *L. nigrinus* predators. Under UV-A light in the field and laboratory, there are several colors present in hemlock woolly adelgid twig samples that have *L. nigrinus* predation, as this predator is predominantly a hemolymph feeder on adelgids. The progrediens predator *S. tsugae*, consumes nearly the entire adelgid's body contents. The characteristic predation patterns between these two predators can be distinguished using UV; this will be dealt with in a later paper.

It was difficult to accurately photograph the 4 main fluorescent colors we initially saw, using a normal digital iPhone 4S camera in a darkened room. We are still refining this process, using time-lapse photography. We hope to produce more accurate photography as we understand this system better.

A normal, undisturbed adult adelgid's honeydew without predation glows a bluish-white color (Fig. 1). This may be from uric acid present in the honeydew. Adelgid adults damaged by *L. nigrinus* predatory activity are bleeding hemolymph that glows an intense chartreuse (yellow-green) color (Fig. 2). Predator-damaged adelgid eggs glow a bright yellow (Fig. 3). Both *L. nigrinus* and *S. tsugae*'s frass glows a brilliant orange (Fig. 4, 5). We have found this brilliant orange substance's fluorescing color to persist for several weeks; then it fades into a reddish pink prior to disappearing. Thus, we believe the age of predator frass can also be determined by the degree of redness and brilliance present. These 4 main colors (blue-white, chartreuse, yellow, and

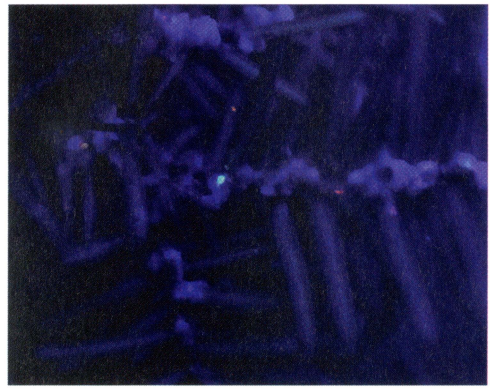

Fig. 1. An undisturbed hemlock woolly adelgid ovisac; the uric acid is glowing blue (center) surrounded by *L. nigrinus*-predated ovisacs, which glow an orange color.

orange), are present on hemlocks with predator activity, making previously invisible predator activity visible to the naked eye in the field and laboratory.

Jones et al. (2012, Naturewissenschaften 99: 583 - 586) have identified 2 of the major fluorescing compounds in the hemlock woolly adelgid as an anthraquinone, chrysophanol, and its anthrone precursor, chrysarobin. They also found that the ratio between these 2 compounds varies throughout the season, with chrysarobin dominating from June until October, mainly in the progrediens; then mature sistens adelgids had chrysophanol dominant by December.

Three closely-related commercially-important coloring products, cochineal (carminic acid), kermes (kermesic acid), and lac-dye (laccaic acid) have been derived from insects belonging to the Coccidæ. These brilliant red dye products contain

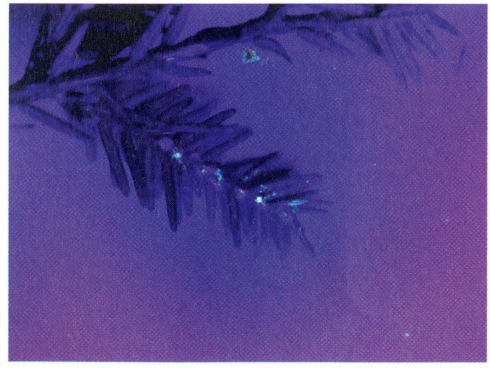

Fig. 2. Damaged hemlock woolly adelgids; their hemolymph glows a chartreuse (yellow-green) color, indicative of *L. nigrinus* predator activity. Photo date: 2 February 2012.

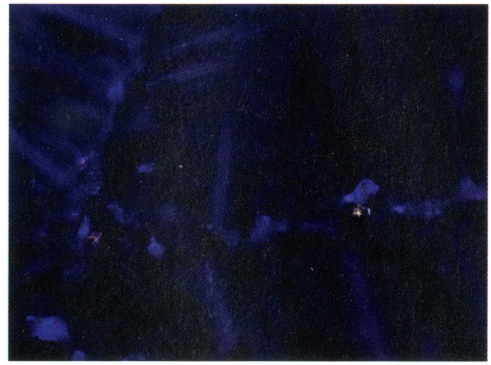

Fig. 3. Damaged Hemlock woolly adelgid eggs in a *L. nigrinus*-predated ovisac glow bright yellow (right); the predator frass to the left of the ovisac at the base of the twig is glowing orange. Photo date: 19 April 2012.

anthraquinones and have been the subject of extensive chemical investigation. The fluorescent properties of certain anthraquinones produced by aphids (known as aphins and aphinins) and other coccidae under UV light were first recognized by both German and British chemists, when pine bark adelgid was accidently introduced into Great Britain from the USA during the 1930s and became commonly known as 'American Blight' (Duewell et al. 1948, Nature 162: 759 - 761). Each of the aphin compounds shows a bathochromic spectral shift under UV-A light with increase in pH (Table 1). The UV fluorescence emission of these aphins simply runs through the visual spectrum from violet to red.

We have been able to use this UV viewing technique alone in the field in the darkness, or UV light viewing under a dissecting scope to detect and illuminate predator

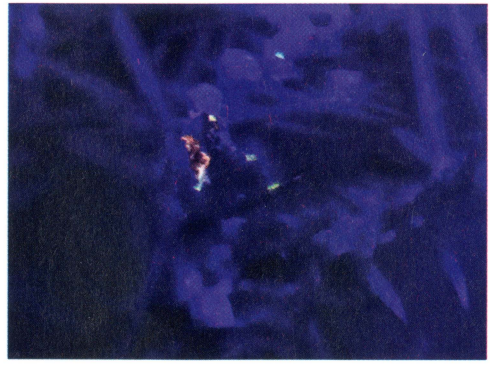

Fig. 4. Hemlock woolly adelgid ovisac damaged by *L. nigrinus* predation, glowing both yellow (egg damage) and orange (*L. nigrinus* frass). In the background is an undisturbed ovisac glowing bright blue. Photo date: 23 April 2012.

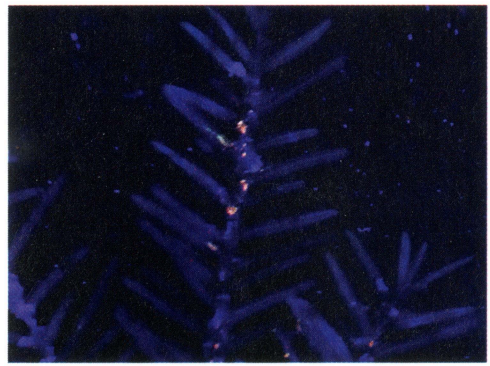

Fig. 5. Frass of the summer predator *Sasajiscymnus tsugae* also glows bright orange and can be used to detect its activity in the field. Photo date: 31 May 2012.

activity without having to physically dissect ovisacs. UV illumination under a dissecting scope allows for a much more elegant and rapid assessment of predator activity. Any chartreuse, yellow, or orange glowing color denote various life stages of adelgid physical damage by predators, and allows us to see predator activity that may have been missed by the naked eye. We can view the activity of the predators, which can be followed through time on the same branch using UV light to show the activity patterns and trail(s) of the predators as they feed.

To avoid unnecessary, redundant chemical treatments for conifers infested with adelgids, UV viewing can be used by commercial entities. We believe this tool can be used by foresters, consultants, arborists and even trained landowners to determine the presence of adelgid predators in a given location at the right times of year to give an integrated approach to biologically-based adelgid pest management.

The fluorescent properties of these compounds also apply to other adelgid species on conifers such as pine (pine bark adelgid) and fir (balsam woolly adelgid), and their predators (syrphids, lacewings, silverflies, predatory midges, predatory beetles, and spiders; RCM personal observations). Further research in conjunction with other researchers will hopefully elucidate the role that these anthrone and anthraquinone compounds play as potential feeding deterrants, feeding attractants and arrestants of a wide variety of adelgid predators across many species of conifers.

Table 1. Colors of the fluorescence of the series of Aphins-*fb* in acid, neutral, and alkaline media under UV-A light.*

Medium	Protoaphin	Xanthoaphin	Chrysoaphin	Erythroaphin
Acid	Dull green	Blue-green	Intense chartreuse	Orange-red
Neutral	Dull green	Blue-green	Intense chartreuse	Orange-red
Alkaline	Dull violet	Brilliant chartreuse	Brilliant chartreuse	Dark ruby-orange

*After Duewell et al. (1948) in: Rockstein et al., 1978, Biochemistry of Insects, p.248.