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FLIGHT PHENOLOGY OF SIREX NIGRICORNIS (HYMENOPTERA: SIRICIDAE) AND OTHER WOODWASPS IN ALABAMA

by
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ABSTRACT
Woodwasps (Hymenoptera: Siricidae) are found throughout the world and play important roles in deciduous and coniferous forests ecosystems. Currently only native Siricidae are known to be present in the southeastern United States, but the Eurasian Siricid species, *Sirex noctilio*, is already established in the northeastern United States and anticipated to spread throughout the United States in the next few decades. In this study, a survey for *Sirex* species was conducted throughout Alabama. Traps were baited with a mixture of kairomones determined to specifically attract siricids. Traps were collected and all specimens were identified to species level. Bark and ambrosia beetles known to be species of concern for forest health also were identified and cataloged, as to determine how native *Sirex* species overlap temporally with beetle populations. Siricid species identifications were later confirmed with molecular techniques.

INTRODUCTION
Woodwasps (Hymenoptera: Siricidae) are an important component of forested ecosystems in the Northern Hemisphere by aiding in the decomposition of dead and dying trees. In North America, 23 species of siricid woodwasps have been recorded, including four introduced species (Schiff et al., 2006). *Sirex noctilio* is one of these invasive species, native to Eurasia and northern Africa, first reported in North America in 2005 from Oswego County, New York, United States (Hoebeke et al., 2005). Subsequently, *S. noctilio* currently is found in the northern States of New York, Pennsylvania, Vermont, Connecticut, Michigan, and in the Canadian province of Ontario. *Sirex noctilio* has the potential to cause significant damage to pine forests of the southeastern United States; based on the economic impact it has historically had in commercial pine plantations in the Southern Hemisphere where it has previously invaded (Olatinwo et al., 2013; Hoebeke et al., 2005).

Sirex noctilio causes damage and the death of pines following oviposition within the xylem, where eggs develop into larvae. During oviposition a symbiotic fungus, *Amylostereum areolatum*, and phytotoxic venom also are injected into the tree, along with eggs, resulting in wood decay providing nutritious material that *S. noctilio* larvae feed upon (Edmonds et al., 2011; Slippers et al., 2006). Fungal spores of *A. areolatum* are carried in the mycangium of adult *S. noctilio* located internally at the base of the ovipositor (Edmonds et al., 2011). Growth of *A. areolatum* restricts water flow in the vascular system of infected pine trees by degrading tissues into a white rot (van der Nest et al., 2012).

Forestry is of significant economic importance in the southeastern United States contributing economic, environmental, social, and aesthetic benefits to these States. The USDA [United States

Department of Agriculture] estimates that 244-million square feet of timber could be killed by *S. noctilio*, with $1.9 billion dollars projected to be lost to local economies (USDA 2008). A study by Olatinwo et al. (2013) predicted that *S. noctilio* could cause up to $48- 606 million losses in the State of Georgia over a thirty-year time period, and Yemshanov et al. (2009) estimated a potential loss of $760 million throughout the whole United States over the same time period.

The potential economic and ecological concerns about the future invasions by non-native *Sirex* spp. into the southeastern United States should motivate an increase in the monitoring and surveillance for siricids in this area. The State of Alabama is specifically at risk of invasion because of the potential for infested wood packing material to enter through the port of Mobile (Yemshanov et al., 2009). The objectives of this study were to 1) monitor and survey native and potentially non-native woodwasp populations in various latitudes across Alabama, 2) determine the length of siricid flight seasons, and 3) survey bark and ambrosia beetle catch in traps to determine if emergence spikes overlap temporally.

MATERIALS AND METHODS

Site Design

Surveys were conducted in three regions across the State of Alabama ranging from Talladega in the north and Andalusia in the south. Sampling was conducted at a total of 33 sites continuously from August 2014 to February 2016. Surveys were conducted in Tuskegee (August 2014- March 2016) and Talladega (Oakmulgee Ranger District) National Forests (February 2015- March 2016), and at Auburn University's Solon Dixon Center in Andalusia, Alabama (February 2015- March 2016). These regions were chosen in order to provide coverage throughout the State of Alabama (Fig. 2.1). All sites chosen were located in mixed species forests, dominated by loblolly pine (*Pinus taeda*) mixed with other pines and a few hardwood species (Fig. 2.2).

Black cross vane panel insect traps (Forestry Distributing Inc., Boulder, CO, U.S.A.) were placed at each site and monitored every two weeks. Traps were hung at a 45° angle from a six foot tall metal pipe driven in the ground, which was curved at the top to elicit hanging traps with ease (Fig. 2.3). Collection cups were attached at the trap bottom to catch insects that were lured to the traps. These collection cups were filled with 250 mL of a 30% propylene glycol solution to preserve specimens. Traps were baited using 8 mL glass vials filled with a mixture of 70% α-Pinene and 30% β-Pinene, previously determined by Simpson and McQilkin (1976) to be optimal for attracting *S. noctilio*. This collection method, for surveying woodwasp populations, was undertaken in accordance with Barnes et al. (2014). Specimens were collected from traps every other week continuously through the entire duration of the trapping survey.

Identification

Specimens of siricids and other insects captured were identified to the species level and curated at the Forest Health Dynamics Laboratory at Auburn University. Specimens were later taken to the Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa for molecular analysis for identification confirmation. Species identifications were determined using Schiff et al. (2006).

By catch beetles that are deemed as species of concern were morphologically identified and cataloged throughout the duration of the trapping survey (Table 2.1). Species of concern include *Hylobious pales, Pachylobious picivorous, Ips* spp., *Hylastes* spp., and several common species of ambrosia beetles. Species were determined using taxonomic keys from Wood (1982).

Statistical Analyses

The mean number of female siricid per trap were analyzed using a repeated measures analysis of variance (ANOVA), in accordance with Hurley et al. (2015). The catch size of each species per week between field sites were analyzed by conducting a factorial ANOVA. The dependent variable was quantity while the independent variable was time. All statistical analyses were run using the SAS program (SAS 9.4, 2013).

RESULTS

A total of 131 woodwasps were collected over the duration of this study, all specimens were females (Fig. 2.7). Six of these wasps were identified as *Tremex columba* L.; three were captured in Tuskegee National Forest (Fig. 2.6), two were captured in Talladega National Forest (Fig 2.8), and the last was captured at the Solon Dixon Center (Fig. 2.5). One *Urocerus cressoni* Norton was collected from Tuskegee National Forest (Fig. 2.6). All remaining wasps (n=124) were identified as *S. nigricornis*.

Captures of *S. nigricornis* in Tuskegee National Forest varied from October 2, 2014 to December 11, 2014 and October 15, 2015 to December 10, 2015, but not significantly (p= 0.5291). In Talladega National Forest (Fig. 2.8), the captures ranged from October 17, 2015 until November 28, 2015. In this locale, *S. nigricornis* capture was significantly higher than *T. columba* capture (p= 0.0346). At the Solon Dixon Center, the only captured female was collected on November 19, 2015 (Fig. 2.9).

The earliest capture of a woodwasp was June 13, 2015 in Talladega National Forest (Fig. 3). This specimen was *T. columba*. The latest recorded emergence in the survey was *S. nigricornis* on December 11, 2014 in Tuskegee National Forest (Fig. 2.7). The only *U. cressoni* captured in the study was collected from Tuskegee National Forest on October 2, 2014 (Fig. 2.7).

Mean capture of *Hylastes* spp. and *Ips* spp. were significantly higher than other bark and ambrosia beetle captures at all three sights. Peaks of these populations occurred earlier in the year than peak *S. nigricornis* flight season (October- December).

Beetle populations were higher than woodwasps by number in all localities. In all cases, beetle populations peaked earlier in the year than *S. nigricornis*.

DISCUSSION

No *S. noctilio* were captured in any traps through the duration of the trapping survey with only three woodwasp species captured in Tuskegee National Forest in 2014. Of these species, only S. *nigricornis* and *U. cressoni* utilize pines as hosts (Schiff et al. 2006).

Sirex nigricornis and *T. columba* had similar flight periods, with the exception of the early emergence trapping of *T. columba* in Talladega National Forest on June 13, 2015. There is little likelihood of competition occurring between these species even though their flight periods overlap; *T. columba* only attacks deciduous hardwood trees (Schiff et al., 2006). The flight season observed in *S. nigricornis* was in accordance with Hartshorn et al. (2016) in Arkansas. The observed emergence period of *S. nigricornis* adult females was slightly longer lasting (into December) than previously reported by Haavik et al. (2013), which conducted their survey in a controlled environment in Louisiana.

Woodwasps capture in 2015 in Tuskegee National Forest was lower compared with 2014, but this difference was not significant. Greater numbers could be due to prescribed burns that were conducted in the spring of 2015. Many of the sites and surrounding wooded areas within the study were burned, so traps had to be removed and reconstructed during this time.

To further address this difference, weather data from neighboring Lee County were obtained, because data from Macon County were unavailable. Monthly average temperature and rainfall data were obtained from the National Oceanic and Atmospheric Administration (NOAA, www.ncei.noaa.gov; accessed 21 April 2016). There was a deep freeze event in December 2014 that effectively ended the flight season, and caused the liquid solution in all of the traps to freeze solid. December 2014 also proved to be much wetter than any of the other months during the trapping survey, with over 45 cm of precipitation accumulating.

Bark and ambrosia populations peaked earlier in the year than *S. nigricornis*, suggesting there would not be direct competition for substrate for rearing larvae. These spring and earlier summer peaks could potentially overlap with *S. noctilio* emergence, if they were to be found in the area. If *S. noctilio* were to be found in the area, a high population density could potentially affect bark and ambrosia beetle populations, as there would be more stressed and dying tree material for beetles to be attracted to. It could be hypothesized that the establishment of *S. noctilio* in an area would exacerbate a beetle outbreak, causing even more tree death in an area.

CONCLUSION

Only females were captured, which also was the case in trapping done in Mississippi by Chase et al. (2014) and is generally the case for all siricid trapping studies (Hurley et al., 2015; Johnson et al., 2013; Coyle et al., 2012). Martínez et al. (2014) suggests that male flight is primality restricted to the tops of the trees in the canopy, where mating occurs. Male siricids do not have the exact temporal emergence period that females do, depending on outside temperature conditions. *Sirex nigricornis* males emerged a predicted two days after females according to the degree day models of Haavik et al. (2013).

To conclude, this study provides a greater understanding of native woodwasp populations, primarily of *S. nigricornis*, in three forests in the State of Alabama. The survey shows that emergence periods of female *S. nigricornis* adults were fairly uniform for the duration of the project, even though the total number of specimens caught differed significantly. Cataloged catch of bark and ambrosia beetle populations in the area suggests that these insects peak in population at an earlier time of the year than *S. nigricornis* populations. Emergence habits and population size

estimates of woodwasps are important details to know in this region, where planted pine forests make up a great deal of the economy. A more intensive and exhaustive collection survey is warranted in the future, as the limited time scale on this project cannot determine the regular population sizes of endemic woodwasps.

Figure 2.1. Map of the State of Alabama, USA, indicating the sites where sampling was conducted from 2014-2016.

Figure 2.2. Intensely managed field site in Tuskegee National Forest that had recently been burned. Black panel trap can be seen in left background.

Figure 2.3. Black cross vein panel trap set in Tuskegee National Forest, with white collection cup on bottom.

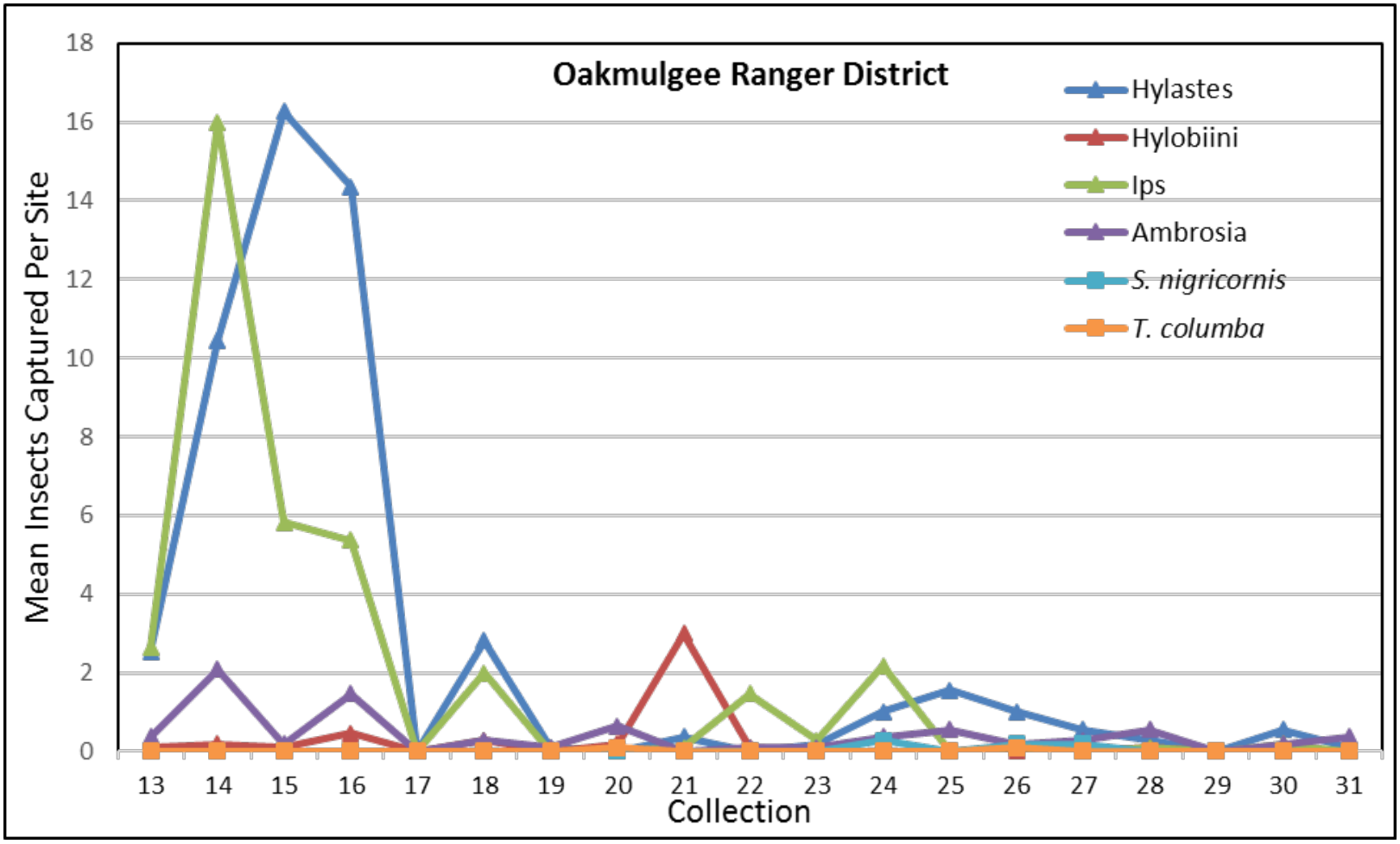

Figure 2.4. Mean insects captured at the Oakmulgee Ranger District of Talladega National Forest. *Ips* spp. and *Hylastes* spp. peak early in the summer, before Hylobiini or *S. nigricornis* in the fall. Collections from 3/7/15 to 1/20/16.

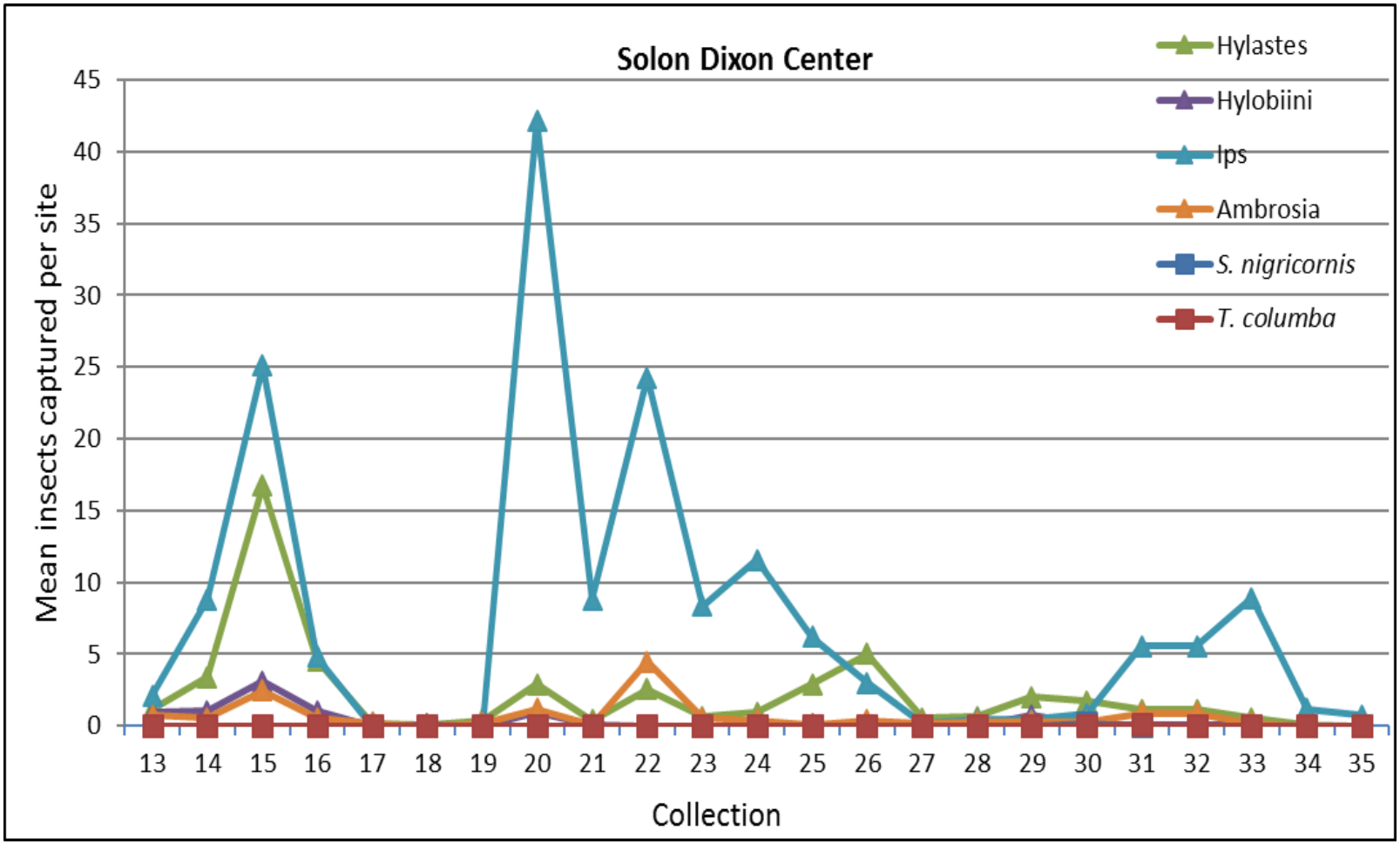

Figure 2.5. Mean capture of species of concern per site in Solon Dixon. *Ips* spp. and *Hylastes* spp. mean catch per site is significantly higher, and occurred earlier in the summer than more northern sites in Tuskegee and Oakmulgee. Collections from 3/11/15 to 1/28/16.

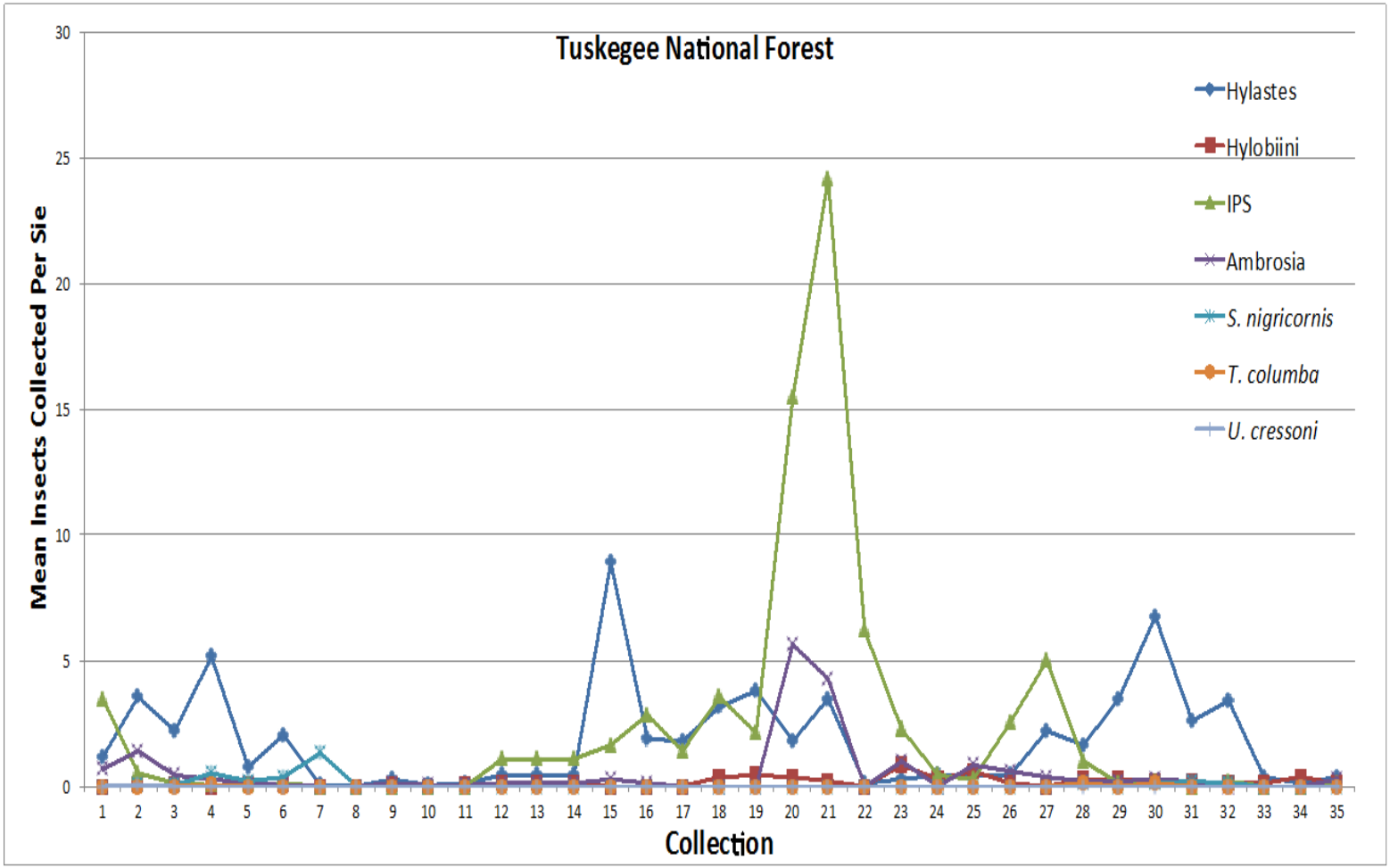

Figure 2.6. Mean capture of insects of concern per site in Tuskegee National Forest. Mean *S. nigricornis* capture significantly lower than *Ips* spp. and *Hylastes* spp. Peaks in these two genera occur during mid-summer, before *S. nigricornis* emergenc

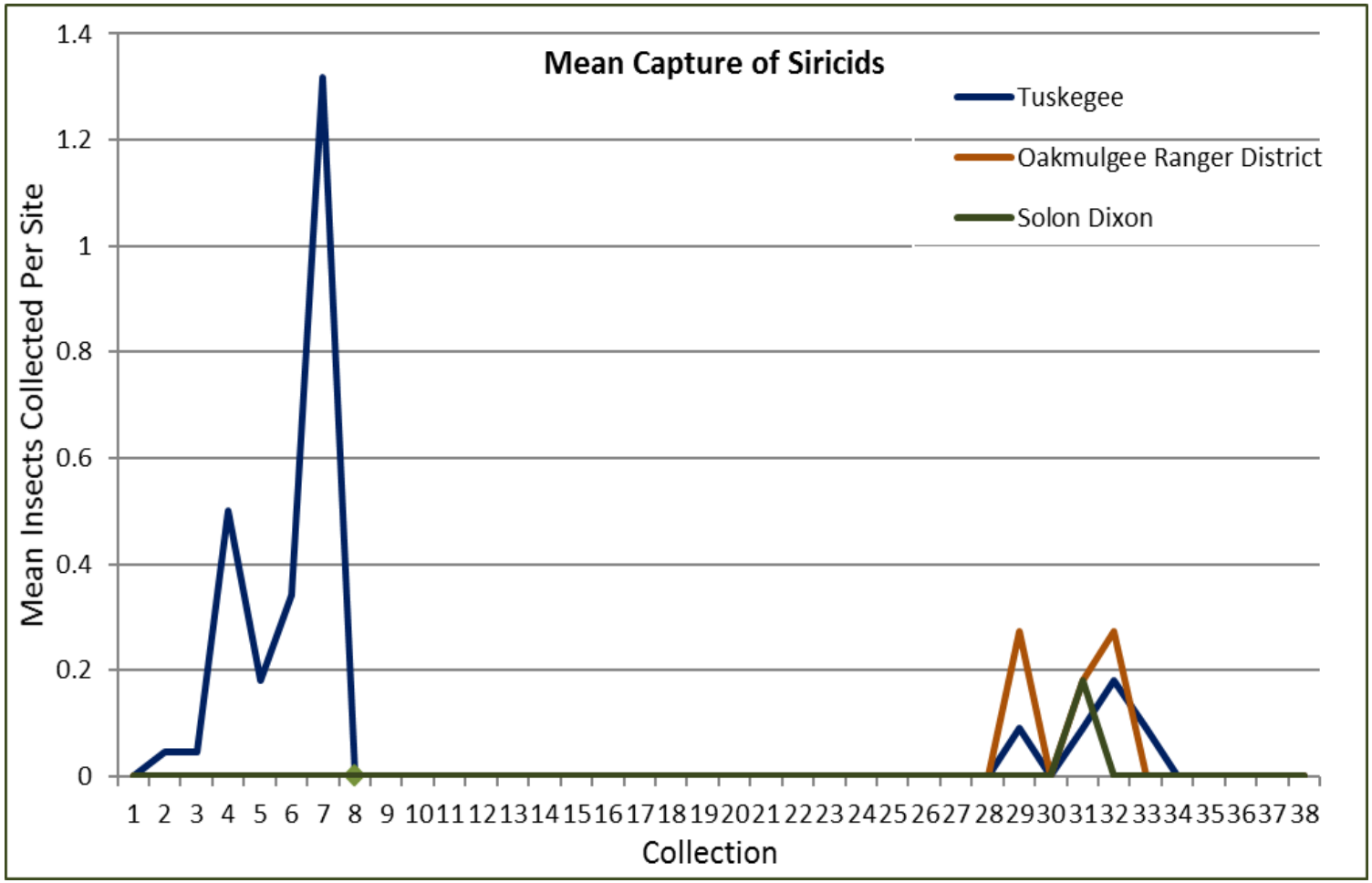

Figure 2.7. Mean capture of siricids per site in from September 2014 to March 2016. *S. nigricornis* peaks are between 10/30/14 and 12/11/14, then 10/15/15 to 12/10/15.

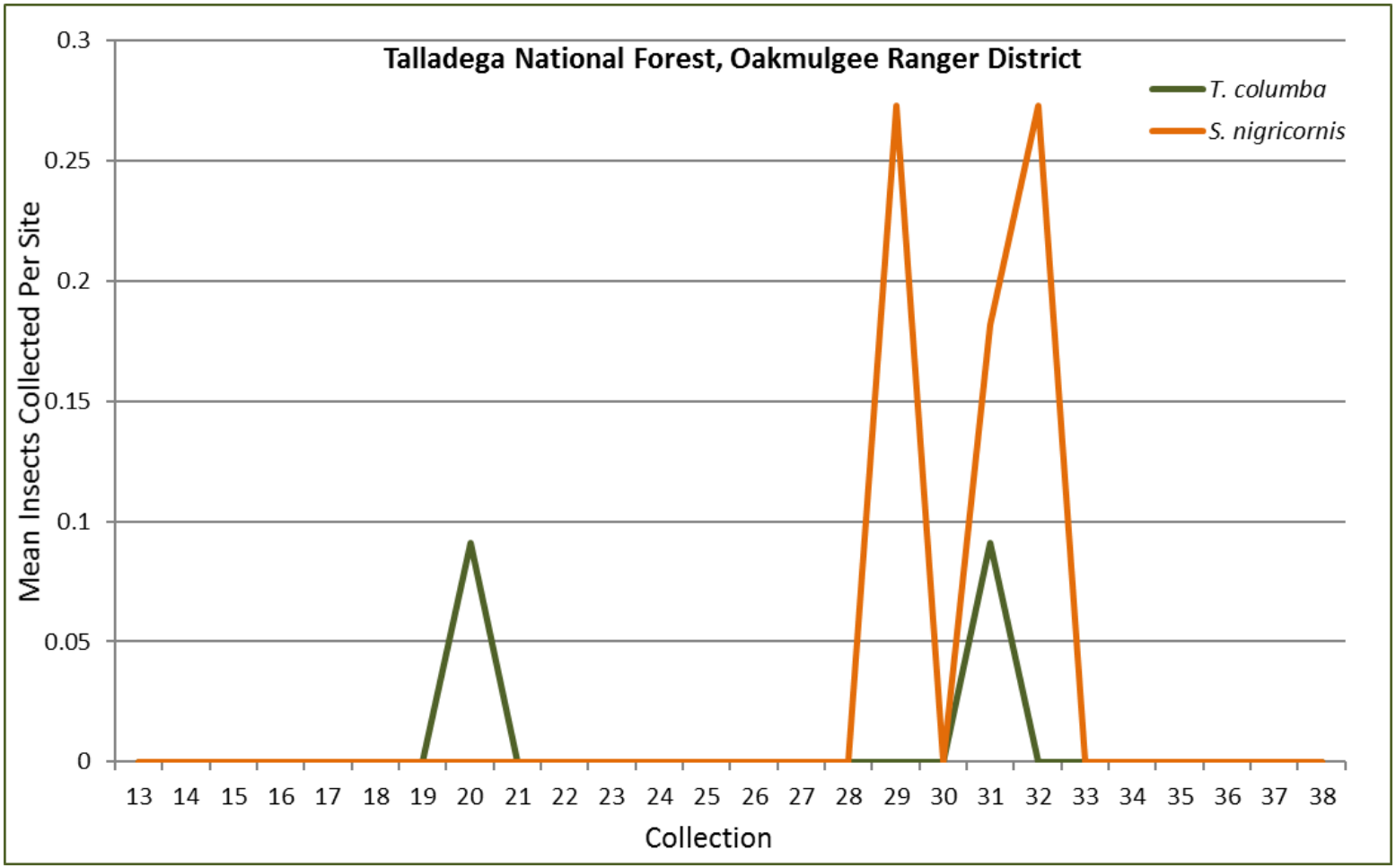

Figure 2.8. Mean capture of siricids per site in Oakmulgee Ranger District of Talladega National Forest. *S. nigricornis* peaks are 10/17/15 and 11/28/15.

Table 2.1. Species of concern captured as by-catch and cataloged throughout the duration of the study.

Designation in Graph	Species
Hylastes	*Hylastes salebrosus, Hylastes porculus, Hylastes tenuis*
Hylobiini	*Pachylobius picivorous, Hylobious pales, Pissodes nemorensis*
Ips	*Ips avulsus, Ips grandicollis, Ips calligraphus*
Ambrosia	*Xyleborus pubescens, Xyleborus ferrugineus, Orthotomicus caelatus, Xyleborinus saxeseni, Xylosandrus crassiusculus, Xylosandrus compactus, Xylosandrus germanus, Xyleborus mutilates, Monarthrum fasciatum, Monarthrum mali, Pityoborus comatus, Trypodendron scabricollis, Dryoxylon onoharaensum*