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Cover Page Footnote

The authors would like to thank Dr. Eric Lovely at Arkansas Tech University for helping with moth identification. We also want to thank the Arkansas Tech University Undergraduate Research Office for funding this project through an undergraduate research grant. Field work would have been impossible without the help of multiple technicians, and the authors thank Stetson Collard, Donna Curran and Amber Steele for their tireless efforts in the field.

The Impact of Prescribed Fire on Moth Assemblages in the Boston Mountains and Ozark Highlands, in Arkansas

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Running title: Prescribed Fire Impacts Moth Assemblages

Abstract

In addition to the impacts of prescribed fires on forest vegetation, this ecosystem process also has dramatic impacts on associated insect assemblages. For herbivorous, terrestrial insects, fire predictably results in a cycle of initial insect population reduction followed by recovery and growth, in which these insect populations exceed pre-fire abundances. We sought to examine if fire-induced disturbance cycles make prescribed burned areas more or less suitable specifically for moths (order Lepidoptera), which is a major food source for, among others, multiple bat species. We surveyed moth assemblages at 20 burned and 20 unburned sites in the Boston Mountain and Ozark Highland ecoregions of Arkansas, to determine if biomass or abundance of moths differed between areas that had been burned in the past 10 years, and those areas that had never been burned. Samples were collected early (April to July) and late (August to November) in the growing season of 2017 (hereafter early season and late season, respectively). We compared biomass and abundance of all moths, and of five representative moth species, between burned and unburned sites. The five moth species were chosen and considered to be representative due to their high relative abundance, and ease of identification. The five chosen moth species included the banded tussock moth (*Halysidota tessellaris*), white-dotted prominent moth (*Nadata gibbosa*), ailanthus moth (*Atteva aurea*), grape leafroller (*Desmia funeralis*), and painted lichen moth (*Hypoprepia fucosa*). Results from paired t-tests showed no significant difference in total biomass, or abundance of representative species between burned and unburned sites. However, generalized linear regression models showed significantly higher abundance of moths in areas with high basal area that had been previously burned ($\beta = -0.038 \pm 0.004$ SE, $p < 0.0001$). Lower number of snags ($\beta = -0.081 \pm 0.0044$; $p < 0.0001$) and more open canopy ($\beta = 0.001 \pm 0.0001$ SE; $p < 0.0001$),

also increased abundance of moths in an area. Our results show that fire acts as an intermediate disturbance, driving moth populations in the Ozark Mountains of Arkansas.

Introduction

Fires, Then and Now

Fire is important in natural ecosystems. Fire regulates competition of vegetative communities, consumes litter and debris, cycles nutrients into the soil, controls insect pests and diseases, and facilitates fire-dependent species (USDA Forest Service Southern Region 1989). The history of the Ozark Mountains of Arkansas and Missouri exemplifies the importance of fire in a hardwood context. For the purpose of this study, we will use the term, "Ozark Mountains" to refer to the mountainous region encompassing the Boston Mountain and Ozark Highlands ecoregions. Studies on fire scars of trees in the Ozark Mountains revealed that on average, fire intervals for the study site occurred every 7.7 years from 1670-1820 but decreased to every 2 years from 1821-1880 (Stambaugh and Guyette 2006). Native peoples regularly used fire to clear land and limit vegetative growth, as did European settlers arriving in the 1800s (Waldrop and Goodrick 2012). Historic accounts exist that show the structure of forests, including the Arkansas Ozark Mountains, in the early 1800s when fires were frequent. Land surveyors of the time recorded that the Ozark Mountains were characterized by open woodlands and prairies (Foti 2004; Stambaugh and Guyette 2006; Jacobson and Primm 1997). As a source of ecological disturbance, fires kept these woodlands and prairies from maturing into dense, closed canopy forests.

Humans began to suppress fires in the 1900s. Fire-scar studies reveal that some sites in the Ozark Mountains had not been burned since 1972 (Guyette and Spetch 2003; Guyette *et al.* 2006; Stambaugh and Guyette 2006). Fire suppression has caused a large

increase in tree density and allowed for the accumulation of leaf litter and debris in environments such as the Ozark Mountains. These closed-canopy forests offer less foraging opportunities for wildlife than the open woodlands and prairies that were present in the past (Ober and Hayes 2008; Bender *et al.* 2015). Although the effects of prescribed burning are not fully understood, natural resource agencies are using prescribed burns in order to restore forests to some of their former conditions (Dey and Hartman 2005).

Insects

Insect abundance declines during fires (specifically terrestrial insects), and for approximately two months after, due to the initial fire exposure, the loss of food sources, and the loss appropriate habitat (Swengel 2001). Mild surface fires consume vegetation, cause shoot dieback, and can kill trees 12 cm in diameter and smaller that are used by insects to live in and feed on (Dey and Hartman 2005). In addition, fire burns leaf litter that some insects live in and consume. The majority of the negatively impacted terrestrial insects recover within one year, and the rest recover within two years after a fire, after which these insect populations grow to exceed pre-fire numbers (Dajoz 1998; Swengel 2001; Evans *et al.* 2013). Thus, fire yields a cycle of insect population declines followed by periods of recovery and growth. It is not currently known if this cycle, which results from prescribed burning, makes burned areas more or less favorable for terrestrial insects than unburned areas over the long-term. This study seeks to determine if the effects of fire make prescribed burned areas more likely to support higher moth abundances, specifically compared to areas that had not been burned recently.

Whether moths are in higher abundance in burned areas or unburned areas may depend on their plant hosts' resilience to fire. We were interested in five moth species, which included: ailanthus moth (*Atteva aurea*), painted lichen moth (*Hypoprepia fucosa*), grape leaf folder (*Desmia funeralis*), white-dotted prominent moth (*Nadata gibbosa*), and the banded tussock moth (*Halysidota tessellaris*). Of the moth species chosen for study, *H. fucosa* relies only on lichen and mosses that typically recover slowly after fire. *Halysidota tessellaris* uses multiple woody species, including alder (*Alnus* spp.), ash (*Fraxinus* spp.), and oaks as host plant species (Beadle and Leckie 2018). *Nadata gibbosa* relies on oaks, maples (*Acer* spp.) and cherry trees (*Prunus* spp.), among others, as hosts, whereas *D. funeralis* is associated with evening primrose (*Oenothera* spp.), grape, and redbud trees (*Cercis canadensis*) (Beadle and

Leckie 2018). *Desmia funeralis* also relies on vegetative hosts such as grasses and flowers that proliferate after fire. Lastly *A. aurea*, depends primarily on ailanthus trees as hosts (Beadle and Leckie 2018), these trees are known to be hardy and shade-tolerant, and although not fire-resistant (Rebbeck *et al.* 2014), the plant has a tendency to regenerate quickly after fires (Fryer 2010).

Species of trees with thick bark can protect and insulate themselves from fire and tend to be fire resistant. Trees that have thick bark and are more fire resistant include *Juglans nigra* (black walnut), *Pinus* spp. (pines), *Salix nigra* (black willow), *Diospyros virginiana* (American persimmon), *Celtis* spp. (hackberry), *Crataegus* spp. (hawthorn), *Rhus* spp. (sumac), and *Quercus* spp. (oaks) (Onduso 2013, Karstenson 2010a). Fire resistant understory vegetation includes *Vaccinium* spp. (blueberry), *Rubus* spp. (blackberry), *Trifolium* spp. (clover), and *Vitis* spp. (grape) (Onduso 2013; Karstenson 2010a). Moth species that rely on fire resistant hosts such as those listed were expected to be present in higher abundance in burned sites or show no difference in prevalence between burned and unburned sites.

Since fire consumes litter and debris, and helps limit vegetative competition, it is expected that fire would allow groundcover such as grasses and flowers to proliferate. This could affect a moth species such as *D. funeralis* that relies on some grass species as host plants. In a high-intensity fire-study, grasses recovered within two to three years after the fire, and grass cover was much higher five years after the fire than it had been prior to fire (Ivanova *et al.* 2017). Moth species that rely on vegetative hosts are therefore expected to be more numerous in burned areas. For those moth species that rely on lichen and mosses as hosts, we expected the opposite trend. Lichens and mosses tend to decrease in fire-prone areas, with slow recovery after fire (Garrido-Benavent *et al.* 2015; Ivanova *et al.* 2017).

Due to a need for further investigation into the impacts of fire on forest moth communities, we chose to compare burned and unburned sites across the Ozark Mountains. We predicted that moth species which have fire resistant hosts would either show no significant difference in abundance in either burned or unburned sites, or show a preference for burned sites. Moth species with fire-susceptible hosts were predicted to have higher abundance in unburned sites. In general, since moths have been shown to recover after fires and exceed pre-fire numbers, moth biomass and abundance were hypothesized to be higher in the burned sites. To test this hypothesis we collected and compared moth samples from twenty burned and twenty unburned sites

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in the Ozark Mountains of Arkansas.

Methods

Location

Data was collected in the Ozark Highlands and Boston Mountain ecoregions in the Ozark Mountains of Arkansas. Elevation ranges from 122 m at the confluence of the Buffalo and White Rivers to 730 m in the Boston Mountains. The Ozark Mountains have a mesic temperature regime and receive 107 to 127 cm of precipitation per year (Karstenson 2010a; Karstenson 2010b). The region is characterized by second-growth forests recovered from timber and agriculture industries (Jacobson and Primm 1997). Lowland areas also include livestock farming and pastureland (Karstensen 2010a; Karstenson 2010b). Prescribed burns are implemented in the area, by the National Park Service, partly to increase numbers of valuable oak species. Oaks respond well to fire due to reduced competition and decreased canopy cover. The Ozark Mountains hardwood forests primarily consist of oak, short leaf pine (*Pinus echinata* spp.), beech (*Fagus* spp.) and hickory (*Carya* spp.) (Onduso 2013; Guyette *et al.* 2006).

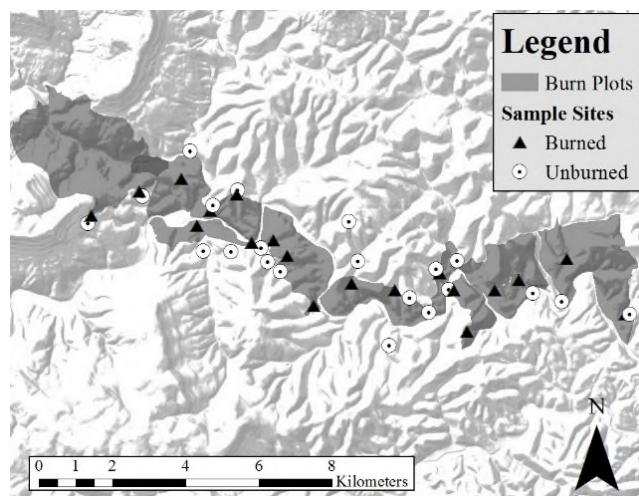


Fig. 1. Study area in Newton County, AR showing the forty sites surveyed and sampled from April–November 2017. The gray area is the prescribed burned area, the triangles are the burned sites, and the circles are the unburned sites (adapted from Blanco 2018).

Site Variables

Forty sites in the Erbie, Ozark, and Pruitt areas in the Buffalo National River, managed by the National Park Service, were chosen for sampling (Figure 1). Twenty of these sites have undergone prescribed burning, in the previous 3–5 years, by the National Park Service, and 20 of these sites have no record of being

burned in the previous 10 years. All of the sampling sites were chosen at random. Each was located no less than 30 m from any road and was at least 1.5 km from any adjacent sites. Basal area, canopy cover, and the number of snags were all measured or recorded at each of the 40 sites. These variables were measured in a circular plot with a radius of 11.5 m at each site. Canopy cover was assessed using a spherical densiometer, at the plot center and in the four cardinal directions from the plot center, recorded as the percent “openness” of a site, and then averaged for each site. The total number of snags with a DBH of at least 16 cm were recorded in each of the 11.5 m circular plots.

Moth Collection

Moth samples were collected from April to November of 2017. This period was divided into two sampling intervals, and labeled accordingly as: early season (from April–July), and late season (from August to November). The aim was to sample each of the 40 sites once during each season; 35 of the sites were successfully sampled twice. Each site was sampled one time per season, and a total of two sites were sampled each night, one at an unburned site, and one at a burned site, for a total of 70 nights of moth sampling.

A Universal Black Light Trap with a 12-watt black light powered by a 12-volt battery was used to attract and trap all insects (Bioquip Inc., Rancho Dominguez, CA). A glass jar containing methyl acetate covered by a mesh cloth was used in the traps to kill the insects. Each trap was hung on a 1.5 meter-tall, metal shepherd’s hook at a random point in the circular plot (Threlfall *et al.* 2012). The trap was set 30 minutes before sunset and taken down 30 minutes after sunrise. Batteries were tested to ensure longevity of the light source before each trapping event.

Sample Processing

After collection, the insect samples were stored in brown paper bags and then dried at air temperature for at least 4 days (samples were weighed every day, until weight stopped changing). All samples were reweighed every day following the initial drying period to ensure that mass stayed the same. After samples were dried moth species were separated from all other insects, weighed, and counted. The total moth biomass from any given trap was recorded (g). The five moth species that we were specifically interested in were separated from the combined insect and moth samples and counted, and weighed, as individual species.

Data Analysis

Statistical analyses were performed to compare total biomass of all moths, total abundance, and the abundance of the five representative species, between burned and unburned areas, and between early and late season. To investigate differences in abundance of the five selected moth species between the early and late season, a paired t-test was conducted (abundance from each species from each trap for each season, and for burned and unburned sites were compared). To compare total moth abundance and biomass among sites, a generalized linear regression model was developed. Errors associated with repeated measures (non-convergence of models) forced us to average data from the two sampling seasons; we then compared burned and unburned areas. Two generalized linear regression models were used to compare total moth abundance and biomass, each as a response variable, between burned and unburned areas. Each of the models included burn status (factor with 2 levels), basal area (continuous), number of snags (counts), and canopy cover (%) as explanatory variables. A Poisson distribution was used for count data. All statistical analyses was done using program R (R Core Team 2016).

Results

After accounting for bad weather, we ended up with 70 nights of insect trapping (35 trap nights in each of the seasons). Biomass of all moths, for both sampling seasons, was 362.55 g. Moth abundance varied between the 2 sampling seasons, with 13,471 individually countable moths in the early season and 6,613 individually countable moths in the late season. Of the 5 species of interest, in both burned and unburned areas, *H. fucosa* accounted for the most individuals (257 and 176 individuals respectively). Two of the moth species (*H. tessellaris* and *N. gibbosa*) were captured in slightly higher, albeit non-significant numbers, in unburned sites than burned sites ($t_{10} = 0.53$, $p = 0.606$; Figure 2). *Atteva aurea* accounted for the lowest recorded abundance for both burned and unburned areas (15 and 16 respectively). There was no statistically significant difference in abundance of any of the individual moth species between early season and late season ($t_{10} = 1.9$, $p = 0.08$; Figure 3).

Total biomass was similar between burned and unburned sites (175.7 g and 174.5 g respectively), resulting in no statistically significant differences, and high standard errors (unburned areas: 0.337 ± 0.814 g, $p = 0.681$).

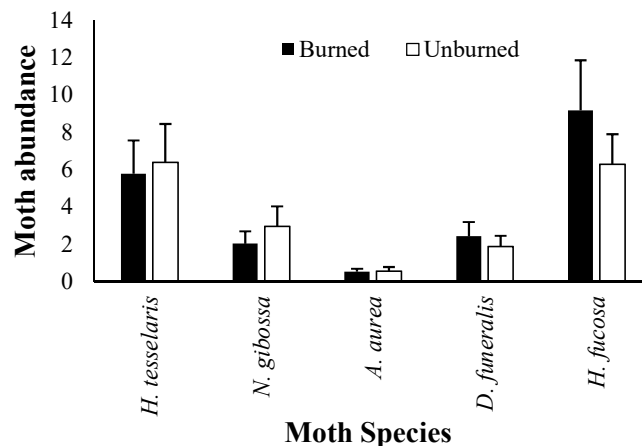


Fig. 2. Average abundance +SE of 5 representative moth species in burned sites compared and unburned sites. Data for the early summer season and late summer season is summed. Moths collected April-Nov 2017 in Newton County, AR.

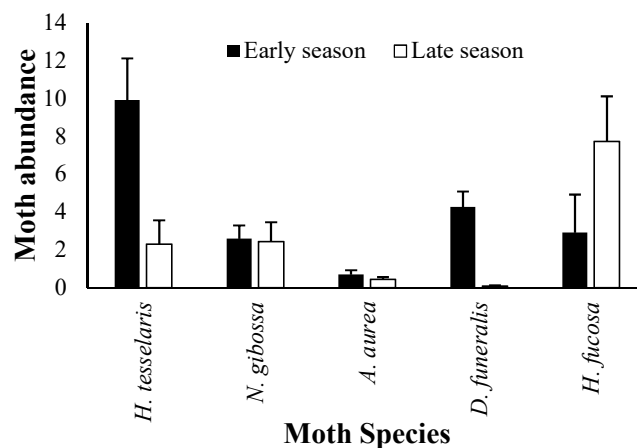


Fig. 3. Average abundance +SE of 5 representative moth species in the early sampling season and late sampling seasons. Data burned and unburned areas is summed. Moths collected April-Nov 2017 in Newton County, AR.

Total moth abundance was lower in unburned areas ($\beta = -0.022 \pm 0.28$ SE; Table 1), than in burned areas. Although these unburned areas generally had higher basal area, more moths were found at sites with higher basal area ($\beta = 0.038 \pm 0.003$ SE; Figure 4). On average burned sites had lower basal area (3.4 ± 0.51 SE m^2/ha) than unburned sites (6.3 ± 1.43 SE m^2/ha) (Figure 5). Hence, although moths were more abundant in burned sites, they specifically had higher numbers at sites with greater basal area.

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Table 1. Model output for the generalized linear model describing variation in moth abundance at 40 sites Newton County, AR. Variables presented in the model included basal area, number of snags, percent canopy cover, and whether or not a site was burned. Moths were collected April–Nov 2017 in Newton County, AR.

Variable	Estimate (β)	p-value
Basal area	0.039 ± 0.004	<0.0001
Snags	-0.078 ± 0.004	<0.0001
Canopy cover	0.001 ± 0.001	<0.0001
Unburned	0.442 ± 1.660	0.438
Unburned * basal area	-0.034 ± 0.004	<0.0001

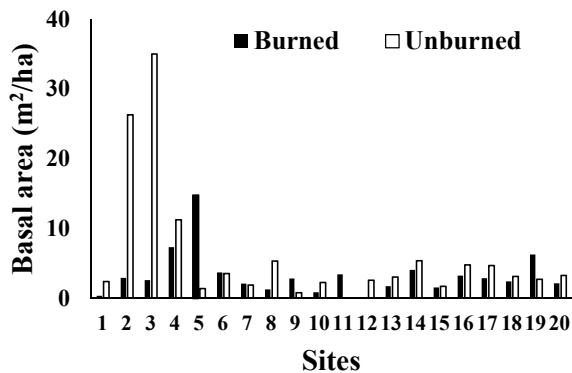


Fig. 4. Basal area (m²/ha) of burned sites compared to unburned sites. Data collected in April–November 2017 in Newton County, AR.

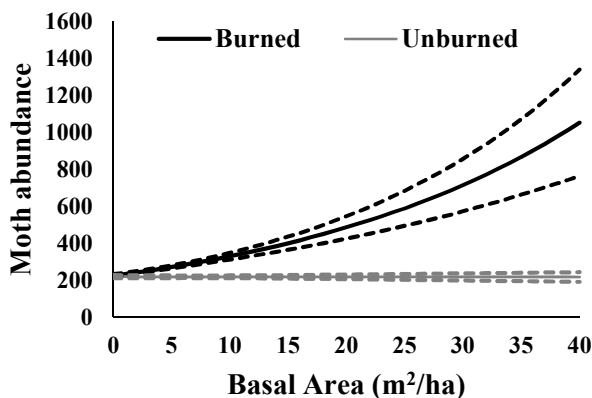


Fig. 5. Predicted moth abundance (solid lines) \pm 95% CI (broken lines) with increased basal area in burned and unburned areas (while keeping canopy cover and number of snags constant). Data collected in April–November 2017 in Newton County, AR.

Burned sites had more open canopies than unburned sites (74% open vs. 47% open; Figure 6), and our regression models showed that canopy cover played a

significant role in predicting moth abundance; more open canopy areas generally had a higher abundance of moths ($\beta = 0.001 \pm 0.0001$ SE; $p < 0.0001$).

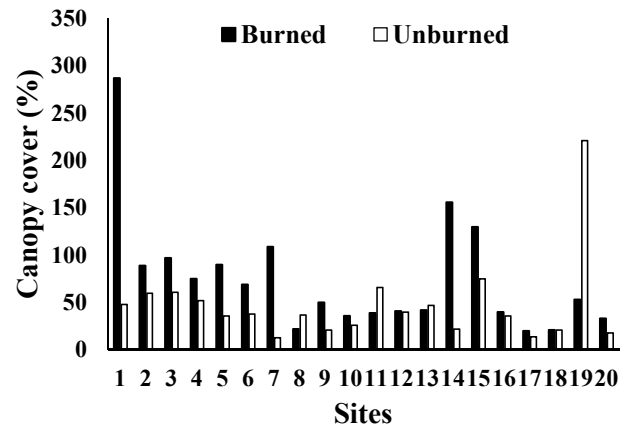


Fig. 6. The canopy cover (%) of burned sites compared to unburned sites. Data collected in April 2017 in Newton County, AR.

Although burned sites generally had more snags than unburned sites (3.1 ± 0.48 SE and 1.7 ± 0.40 SE respectively), moth abundance was lower at sites with high snag numbers ($\beta = -0.081 \pm 0.0044$; $p < 0.0001$) (Figure 7).

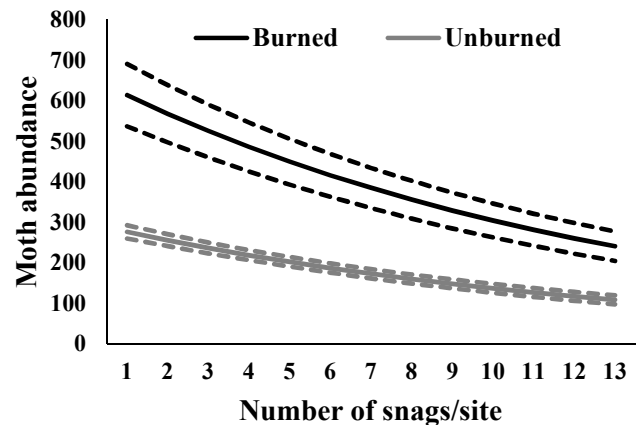


Fig. 7. Predicted moths abundance (solid lines) \pm 95% CI (broken lines) with increased number of snags in both burned and unburned areas, from data collected April–November in Newton County, AR.

Discussion

Similar abundances of *H. tessellaris*, *N. gibbosa*, and *D. funeralis* in burned compared to unburned sites was expected. These numbers can be explained by the

fact that these species all have fire dependent hosts that likely did not differ between burned and unburned sites (Onduso 2013). The lack of difference in abundance of *H. fucosa* between burned sites and unburned sites suggests that prescribed fire in the Ozark Mountains does not significantly alter the moss and lichen stratum that *H. fucosa* relies on. The prediction that *H. fucosa* would prefer unburned sites because mosses and lichen decline after fire and recover slowly was not supported (Garrido-Benavent *et al.* 2015; Ivanova *et al.* 2017). This may be because the Ozark prescribed burns are too mild to significantly impact the moss and lichen stratum. Prescribed burns in the Ozark Mountains are low intensity surface fires, the least destructive type of fire (Dey and Hartman 2005; Onduso 2013). Surface fires like those used in prescribed burns do not always completely burn a site and can leave some trees unscarred (Guyette and Spetich 2002). Mosses and lichen may therefore remain in unburned patches, within the burned areas, providing resources for *H. fucosa* (Reinhard and Menges 2004; Calabria *et al.* 2016).

The fact that there was not a decrease of *A. aurea* in burned areas could indicate that the decrease in litter through prescribed fires favors the establishment of small, light seeds such as those of ailanthus trees (Onduso 2013). Although burning is frequently used to control some invasive species, it can often lead to the spread, and increase, of alien invasive plant species (Grace *et al.* 2001; Guthrie *et al.* 2016). In our case fire did not affect *A. aurea*, which could be indicative of fires not having an impact (positive or negative) on the ailanthus tree. Of course, more detailed analyses of other moth species would yield clearer results. Ideally we would have wanted to examine moth species that are specifically fire-sensitive or fire-resistant (either physiologically or because of characteristics of their plant hosts), but in the end ease of identification and larger numbers guided us to use the species that we have.

The lack of difference in biomass, but much higher abundance of moths, in prescribed burned sites indicate that prescribed burns in the Ozark Mountains are impacting moth assemblages. It is known that the density of generalist butterflies is higher in pine-oak barrens where burning occurs than in idle pine-oak barrens (Swengel and Swengel 2001). Prescribed burns in the Ozark Mountains are a management tool used to promote growth of beneficial herbaceous plants and to discourage growth of herbaceous plants that have little value to wildlife (Dey and Hartman 2005; Onduso 2013). Moth abundance may be higher in prescribed burned sites because prescribed burns encourage growth

of vegetation, providing a food source for moths (Ivanova *et al.* 2017). Terrestrial insects (and specifically herbivorous insects) are known to frequent areas that have recently been burned, in preference over unburned areas, specifically to take advantage of new vegetative growth (McCullough *et al.* 1998; Swengel 2001). The low intensity, and infrequent application, of prescribed burns in the Ozark Mountains may not alter habitats enough to significantly change biomass of all moth species, but could positively affect specific moth species. This implies that perhaps specific lighter weight moths increased in abundance, and compensated for the loss of some of the heavier species. A future study should examine numbers of all moth species, and how those differ between burned and unburned areas.

Although forest-stand characteristics, such as basal area, play a key role in insect abundances and diversity, Dodd *et al.* (2008) showed that short term changes to these characteristics only alter moth assemblages temporarily and that of more important concern should be overall woody plant species richness. Dodd *et al.* (2012) found a negative relationship between Lepidopteran abundance and basal area, they also showed that Lepidopteran abundance was negatively correlated with disturbance. If fire acts as a large disturbance, the assumption is that there would be a decrease in Lepidoptera abundance or diversity, or both. Our data showed that moths responded positively to burned areas, and more so positively to areas with high basal area within burned stands.

Many moth species prefer early successional, open canopy, habitats (Grand and Mello 2004; Nöske *et al.* 2008), which is in line with our results, and pointed to characteristics of some of the burned areas. The main effects from burns may be an increase in the number of snags, a forest canopy that is more open, or a forest stand that is restored to an earlier successional stage.

The results from the present study support the intermediate disturbance hypothesis proposed by Connell (1978). The mild or intermediate disturbance caused by prescribed burns may be ideal for supporting higher moth numbers. Disturbance caused by prescribed burns may decrease basal area, increase the development of new snags, and open canopy cover (Drapeau *et al.* 2002; Boyles and Aubrey 2005; Peterson and Reich 2011). Without disturbance from prescribed burns, competition may be more intense for moths in unburned sites. Burned sites may be ideal habitats for moths because prescribed burns encourage the growth of vegetation that serves as a food source and habitat for moths. In addition, prescribed burns may be an agent of intermediate disturbance that decrease resource

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competition among moths and allow for greater moth abundance.

Acknowledgments

The authors would like to thank Dr. Eric Lovely at Arkansas Tech University for helping with moth identification. We also want to thank the Arkansas Tech University Undergraduate Research Office for funding this project through an undergraduate research grant. Field work would have been impossible without the help of multiple technicians, and the authors thank Stetson Collard, Donna Curran and Amber Steele for their tireless efforts in the field.

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