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ABSTRACT

The ecology of organisms that co-evolve within an ecosystem is likely to be distinct from that involving organisms recently introduced into an area. To better understand the relationship of earthworms with endophyte-infected tall fescue, earthworms in novel and toxic endophyte-infected tall fescue pastures were enumerated and identified as adults or juveniles. We hypothesized that differences in endophyte infection of the fescue would influence earthworm abundances. Earthworms in two toxic and two novel endophyte-infected tall fescue fields in Fayetteville, Ark., were sampled weekly from January through July 2007. Each type of endophyte-infected pasture was established in 1997 and 2003. Sampling was carried out utilizing a physical dig-and-sort extraction method. Although variable, sampling time was a significant factor in the number of adult and juvenile worms collected. Adult earthworm abundances showed a seasonal trend of declining numbers from winter to summer, while juvenile worms showed an increase from winter to summer. Previous studies have shown that endophyte infection of plants can impact soil organisms. In this study, type of fungal endophyte infection did not appear to impact earthworm abundances; therefore, use of novel endophyte-infected fescue in a pasture is not expected to have an impact on the ecology of earthworms.

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MEET THE STUDENT-AUTHOR

Ashley Rashé

I graduated from Cassville High School, Mo., in 2004 and enrolled in the University of Arkansas as an honors student in the fall, choosing Environmental, Soil, and Water Science as my major. Shortly thereafter, I also enrolled in the Crop, Soil, and Environmental Sciences Department's new minor, Wildlife Habitat. I am currently a senior and have been actively involved in many campus clubs such as the Crop, Soil, and Environmental Sciences undergraduate club, the Sierra Student Club, and Alpha Zeta Agricultural Fraternity.

My first year of college, I began working in the Soil Biology and Microbial Ecology Laboratory under the supervision of Dr. Mary Savin. I began conducting research about earthworm abundances and distributions in endophyte-infected tall fescue pastures. This became the research project for my thesis, and this experience so early in my college career was especially beneficial. In 2005, I participated in the Undergraduate Club Poster Contest at the American Society of Agronomy (ASA) annual meeting in Salt Lake City. In 2006 and 2007, I presented my research at the ASA conferences in Indianapolis and New Orleans, respectively.

I have received many grants, scholarships, and honors throughout my four-year education. Some of the grants and scholarships I have received are the Dale Bumpers College Undergraduate Research Grant, Honors College Grant, the Fontaine R. Earl Scholarship, and the Arkansas State Plant Board Intern Scholarship. I was awarded the National Student Recognition Award by ASA in 2007.

The opportunities I have experienced with the Department of Crop, Soil, and Environmental Sciences have allowed me to succeed in receiving a solid education and gain knowledge for my future endeavors. I could not have asked for better professors and classmates than those I had at the Dale Bumpers College of Agricultural, Food and Life Sciences.

INTRODUCTION

Earthworms are keystone species that can substantially impact biological, chemical, and physical properties of terrestrial ecosystems. Epigeic worms reside in the litter layer and surface soil and consume litter. Anecic earthworms pull plant litter from the ground surface and incorporate it into the soil in burrows. Endogeic earthworms live within the soil horizons, constructing both horizontal and vertical burrows.

Earthworms can recycle nutrients by moving litter into soil, facilitating decomposition, and making nutrients plant available (Amador et al., 2005), thereby increasing nitrogen (N) concentration in vegetative tissue (Callaham and Hendrix, 1998). Earthworms also impact ecosystems through physical changes such as altering aggregates and macropore formation, consequently increasing aeration and allowing easier drainage and less runoff. Anecic earthworm burrows have been found to facilitate faster movement of water and chemicals applied directly to the soil surface through the soil matrix (Shipitalo et al., 1999). However, anecic earthworm burrows can also have detrimental effects. Chemicals applied frequently and in large amounts can be transported rapidly in macropores, and are thus not attenuated during filtration through the soil matrix (Shipitalo and Butt, 1999).

Any impact on earthworm ecology is important to ecosystem functioning. Earthworms are generally considered keystone species and beneficial in agricultural systems because they are ecosystem engineers and have a disproportionately large effect on ecosystem functions, such as decomposition and nutrient cycling. Tall fescue (*Festuca arundinacea* Schreb) is a cool-season grass used commonly in pastures in the United States, particularly in humid regions such as the Southeast (Franzluebbers and Stuedemann, 2005). It is infected with a fungal endophyte that helps the plant but is toxic to cattle during times of the year of high abiotic stresses, such as drought or disease prevalence (Humphries et al., 2000). Inputs of alkaloids from the endophyte into the soil have been shown to alter soil carbon (C) structure through reduction of microbial activity (Franzluebbers and Hill, 2005). Other organisms such as earthworms that consume plant litter may also be affected by the toxin production. In addition to affecting the resource quality of litter, the type of endophyte in symbiosis with the fescue could impact earthworms negatively by altering utilization of belowground resources and nutrients by the plant. In turn, this can affect the relationships that plants have with other soil organisms (Omacini et al., 2005).

Geographical distribution of different earthworm populations has important implications for the effects of worms on an ecosystem. For example, non-native (or exotic) earthworms have profoundly altered physical and biogeochemical properties of northern forests. The forests were previously uninhabited by earthworms. Introduction of worms altered carbon and nitrogen pools, as well as caused a complete loss of the forest floor horizon due to increased decomposition. Although decomposition occurs mainly through activity of microorganisms, earthworms drastically altered root distribution and functioning, reduced pools of C and N through hydrologic and gaseous losses and P through hydrologic losses, and affected the activity of the microbial community (Bohlen et al., 2004).

Arkansas is an example of a state where both native and exotic earthworms overlap in distribution; however, earthworm populations in Arkansas are largely unknown. Population identifications have not been published since the 1950's (Causey, 1952 and 1953) and this information is needed for future ecological studies. We set out to enumerate earthworm abundances in one type of managed ecosystem so that we could also later identify species and investigate population distributions.

The objectives of this study were to determine if earthworm abundances were different under novel and toxic endophyte-infected tall fescue pastures, and how abundances in each of those pastures changed seasonally from January to July. Given the potential for changes in litter quality from the presence of toxic versus non-toxic endophyte, endophyte infection of tall fescue was hypothesized to affect earthworm abundances.

MATERIALS AND METHODS

Study site. The study site consisted of four 1.62-hectare pastures growing tall fescue (*Festuca arundinacea* Schreb). The sites were located in Fayetteville, Ark., a northwest region of the state. Two fields were growing novel endophyte-infected fescue (*Neotyphodium coenophialum* Glenn, Bacon & Hanlin) and two were growing toxic endophyte-infected (*Neotyphodium coenophialum*) tall fescue. One pasture of each endophyte-infection type was planted in 1997 and one pasture of each endophyte-infection type was planted in 2003. All sites had minor amounts of crabgrass, yellow foxtail, and bermudagrass.

Worm collection. Worms were generally collected twice a week from January through July 2007. For another portion of this study not being reported here, we had performed a comparison of two different earthworm extraction methods, i.e. chemical expulsion using a mustard solution and a physical dig and sort method. Worm abundances presented here are from the dig and sort method only. From January to the end of May, dig and sort directly followed use of mustard extraction on the same area, so numbers under-represent total abundances during that time period. However, mustard extraction was variable and not very efficient, so abundances are presented as collected following the application of a mustard solution poured onto a 30 x 30 cm^2 area. Worms were collected for 20 to 40 min (Chan and Munro, 2001). The area was then dug to a depth of 20 cm and soil was removed, spread on a tarp, and sifted through by hand to find earthworms. From the end of May to July, only the dig and sort method was used to collect worms. On each sampling date, one to all four pastures were sampled. In each pasture sampled, three plots (30 x 30 cm^2) were sampled along a transect.

In the field, worm abundances were recorded and worms were stored in specimen cups lined with moist paper towels. Upon return to the laboratory, worms were dipped in boiling tap water to kill them quickly. They were then placed in test tubes with 5% formalin for preservation. Boiling minimizes constriction of the earthworms segments. Earthworms were counted and identified by external features as adults or juveniles. Some worms were not intact after collection and preservation. Unless there was enough of the worm to distinguish adult or juvenile, the worm was not identified as either stage.

Data analysis. Averages (per 30 cm^2 or 0.09 m^2 area) were calculated for total earthworm abundances and for numbers of adults and juveniles, and for each endophyte-infection type for each date sampled, regardless of year that the pasture was planted. For each of the datasets (total, adult, or juvenile abundances) from the dig and sort method, two arbitrary linear regression lines were fit simultaneously: one for toxic endophyte and one for novel endophyte-infected fescue. The fitted lines were tested for equality of slopes. We concluded that the slopes were equal, i.e. with a P value greater than 0.05, and therefore we fit a new model consisting of two parallel lines, one for toxic and one for novel endophyte infection. The fitted parallel lines were tested for equal intercepts. If we concluded that the intercepts were equal, then we fit one line to data combining both types of endophyte infection. No adults were found in late June and July, so the values of zero were removed from the dataset used in the regression analysis of adult numbers through time because those data have no variability.

RESULTS AND DISCUSSION

Average earthworm abundances ranged from 0 to 12 earthworms (per 0.09 m^2 area sampled) throughout the study period (Fig. 1). The slopes ($P = 0.69$) and y-intercepts ($P = 0.71$) of earthworm abundances in soil growing toxic versus novel endophyte-infected tall fescue were not different, causing the regression lines to collapse into one (Fig. 1). Statistically, total earthworm abundances in these pastures were not impacted by endophyte infection.

Temporally, total abundances showed a slight, but not significant, linear decrease over time ($P = 0.055$). Results of other studies have shown that sampling during a similar time period, winter through early summer, was most effective for collecting the highest number of worms. For example, Callaham and Hendrix (1997) found the highest earthworm abundances in a forest ecosystem in Georgia in late spring and early summer and lowest abundances in late summer and autumn. Earthworms numbered 75-80 earthworms per m^2 or approximately 7 earthworms per 0.09 m^2. Although abundances were variable among collections in this and other studies, and comparisons of worm numbers among studies, or across years, may be confounded by fluctuations of soil temperature and moisture, abundances were similar to the findings of our study.

Adult earthworm numbers ranged from an average of 0 to 11.3 earthworms (per 0.09 m^2 area sampled) (Fig. 2.) The slopes ($P = 0.97$) and y-intercepts ($P = 0.87$) were the same for both types of endophyte infection and so were collapsed into one line (Fig. 2). The regression line showed a significant linear decrease over time and adult worms were not present in late June and July ($P < 0.0001$, Fig. 2).

Juvenile earthworm abundances ranged from an average of 0 to 10.3 earthworms (per 0.09 m^2 area sampled) (Fig. 3.) Again, the slope ($P = 0.45$) and y-intercept ($P = 0.54$) were the same for both types of endophyte infection, and so were collapsed into one line. In contrast to the trend in adult numbers, the collapsed regression line showed a significantly linear increase over time ($P = 0.0064$, Fig. 3). Therefore, while endophyte infection did not have an impact on either adult or juvenile worm abundances, we did observe apparent time trends in adult and juvenile abundances. The slope for the adult abundances was steeper than that found for juveniles because adult numbers declined to zero in late June, but juveniles were found throughout the study period.

Our data showed that juvenile abundances significantly increased winter through summer. In a study conducted in Georgia under a forest ecosystem, juvenile earthworms were most abundant November through May, and total abundances were greatest in late spring through early summer (Callaham and Hendrix, 1997). Adults were present, but not in as high abundances as juveniles and, similar to our study, adult abundances slowly began to decrease after May. The decrease in soil moisture in this study (data not shown) may partly explain the absence of adult earthworms in late June and July. This seasonal trend may also imply that once adult earthworms have reproduced, contributions to ecosystem functions will be dependent on juvenile earthworm survival and growth.

For research performed in the tall fescue pastures of Northwest Arkansas, the most appropriate times for sampling to collect highest abundances of worms would be January through late June. However, adult worms are necessary to identify earthworm species. Therefore, sampling for identifications should be conducted in late winter through early spring or January through late May, when adults are present.

Information about the effects that plant endophyte infection status has on soil organisms will allow for better land management decisions (Humphries et al., 2000). The difference in endophyte infection among fescue pastures had no significant impact on earthworm abundances in this study. We had expected that type of endophyte infection (toxic versus novel) would impact earthworm populations based on the ecological importance of earthworms in decomposing plant residues. The lack of significant differences in earthworm abundances, of both juveniles and adults, suggests that whether tall fescue is infected with the toxic or the novel endophyte, it will not impact the ecological relationships with earthworms. Our data suggest that use of fescue that is favorable for cattle, i.e. without the effects of the toxic endophyte, is manageable without compromising the integrity of the ecosystem.

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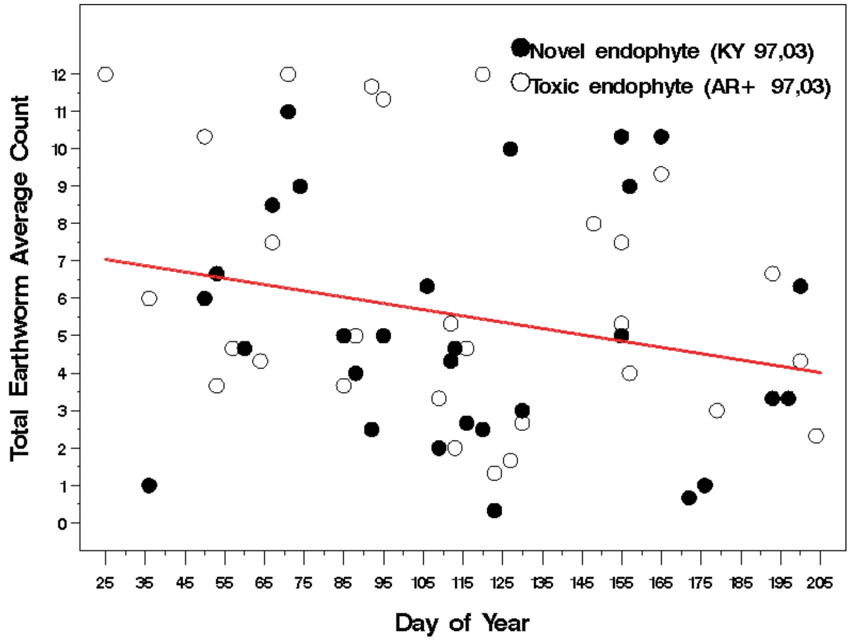

Fig. 1. The average of total earthworm abundances found in novel and toxic endophyte-infected fescue plots from January to July, 2007 (n = 2 - 6). The novel endophyte-infected fescue is indicated as AR+ 97,03 and the toxic endophyte-infected fescue is expressed as KY 97,03.

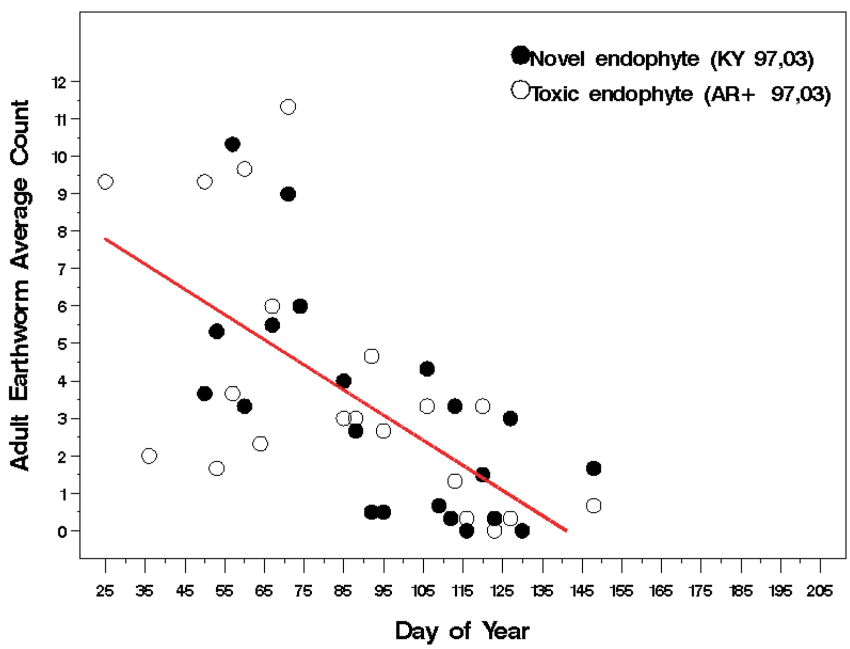

Fig. 2. The average of adult earthworm abundances found in novel and toxic endophyte-infected fescue plots from January to July 2007 (n = 2 - 6). The novel endophyte-infected fescue is indicated as AR+ 97,03 and the toxic endophyte-infected fescue is expressed as KY 97,03.

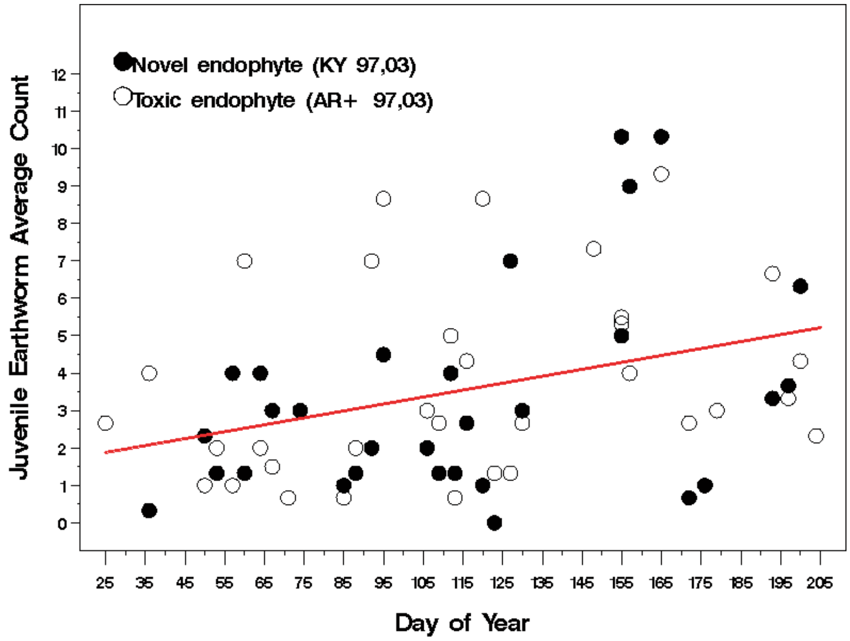

Fig. 3. The average of juvenile earthworm abundances found in novel and toxic endophyte-infected fescue plots from January to July 2007 (n = 2 - 6). The novel endophyte-infected fescue is indicated as AR+ 97,03 and the toxic endophyte-infected fescue is expressed as KY 97,03.