First Confirmed Record and Ecologic Observations of *Ophiocordyceps kimflemingiae* (Hypocreales: Ophiocordycipitaceae) Infecting *Camponotus americanus* (Hymenoptera: Formicidae) in Tennessee[1]

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Abstract Entomopathogenic fungi in the clade *Ophiocordyceps unilateralis* (*s.l.*) parasitize formicine ants (more commonly known as carpenter ants), resulting in fungal fruiting bodies that emerge from the head of the infected ant. This phenomenon is better known as the "zombie-ant fungus." In Oak Ridge, Tennessee, 191 infected ant specimens found on deciduous understory shrubs and saplings were collected from May to December 2022 and from March to May 2023. The diameter of stems on which these infected ants were attached was measured, host plant species were determined, and various morphologic characteristics (e.g., stroma length and perithecial plate diameter) of this fungus were documented. Deoxyribonucleic acid extractions and polymerase chain reaction analysis were conducted to confirm the identity of the fungus. Our results confirmed the presence of a fungal species within the *Ophiocordyceps* clade. *Ophiocordyceps kimflemingiae* was found infecting *Camponotus americanus* Mayr attached to stems of several understory shrubs and saplings, such as *Lindera benzoin* (L.) Blume (spicebush), *Ulmus* spp. (elm), *Acer* spp. (maple), *Betula* spp. (birch), and others. The size of fungal morphologic characteristics varied widely. For example, fungal stroma length ranged from <1 to >30 mm and perithecial plate diameter ranged from as small as 0.1 to >1.5 mm. This research has expanded the known geographic distribution of this fungus, demonstrated fungal morphologic variation, contributed to ecologic knowledge on stem diameter and mandibular death grip of infected ants on deciduous host plants, and expanded the listing of known plant species inhabited by infected ants.

Key Words *Ophiocordyceps*, *Camponotus*, zombie-ant fungus, entomopathogen

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Entomopathogenic fungi have evolutionarily developed manipulative techniques that modify host behavior to ensure fungal survival (Hughes et al. 2010) and increase their rate of infection efficiency (de Bekker et al. 2017). Ophiocordycipitacae (a family of parasitic fungi) currently comprises approximately 140 species, supporting the importance of understanding the taxonomic and functional morphologic variation within this ecologically important fungal group (Evans et al. 2011a). *Ophiocordyceps unilateralis* (*sensu lato*) is a specialized entomopathogenic fungus that

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infects, manipulates, and kills formicine ants (more commonly known as carpenter ants [sub-family Formicinae, family Formicidae]) (Evans et al. 2011a), and is commonly known as the zombie-ant fungus (ZAF). *Sensu lato* is used to describe the species "in the broad sense" because of the growing speciation within the *Ophiocordyceps* genus.

Spores of *O. unilateralis* (*s.l.*) securely attach to the exoskeleton of the carpenter ant before germinating and penetrating it by utilizing a combination of mechanical force and enzymatic secretions (such as chitinase, lipase, and protease) (Evans et al. 2011a). During the process of infection, the fungus produces toxic bioactive compounds that manipulate host behavior (de Bekker et al. 2017), which is termed "zombification." Although the mechanisms for these altered host behaviors are not fully understood, it has been suggested that the fungi are able to turn on or turn off behaviors already known by the host, such as feeding and searching behaviors, rather than creating a completely new behavior (Imirzian and Hughes 2021, Solá-Gracia et al. 2018, Will et al. 2020). This behavioral manipulation causes the ant to climb vegetation (typically understory shrubs) and attach itself to major leaf veins along the abaxial surface by their mandibles, where they remain until death (Hughes et al. 2010). This phenomenon is commonly known as the "mandibular death grip" (Evans et al. 2018) and is directly caused by fungal colonization-induced hypercontracted mandibular muscles (Mangold et al. 2019). Postmortem, *O. unilateralis* (*s.l.*) consumes the inner organs of the ant, and a fungal stroma (stalk-like fruiting body) erupts from the head of the ant. Then, the perithecial plate (which houses fungal spores) forms and later releases fungal spores to infect other ants (Hughes et al. 2010). The manipulative behavior exhibited by *O. unilateralis* is adaptive, meaning the host ant typically attaches itself to vegetation via a mandibular death grip at a distinct location in the canopy that provides optimal humidity and temperature conditions for fungal growth and spore dispersal (Hughes et al. 2010). It has been shown that colonies are unable to detect individuals infected with *O. kimflemingiae* (Imirzian et al. 2020, Solá-Gracia et al. 2018).

One study of a population of insect hosts in Thailand emphasized the host specificity of *O. unilateralis* and concluded that approximately 97% of hosts belonged to the *Camponotus* genus (carpenter ants) (Hughes et al. 2010). Another study (Evans et al. 2011a) stated that even after 150 yr of collecting data, *O. unilateralis* has been found to infect only carpenter ants in southern tropical forests of Thailand. However, *Ophiocordyceps* spp. can infect other genera within Formicidae including *Polyrachis* (subfamily Ponerinae) (de Bekker et al. 2017), suggesting that *Ophiocordyceps* spp. are highly host specific. In addition to host specificity, fungal body morphology has been strongly tied to genetic speciation within the *Ophiocordyceps* genus (Araújo et al. 2014). For example, each ant species is infected by a specific *Ophiocordyceps* spp., where fungal taxonomic names correspond to descriptions of fungal body morphology and the ant species they infect (de Bekker et al. 2017). Thus, species can readily be separated using classic taxonomic criteria including fungal body morphology (Araújo et al. 2018). Although fossil records of *O. unilateralis* date back 23–66 million yr (Hughes et al. 2010), *O. unilateralis* is still, overall, poorly documented, especially regarding its geographic distribution and morphologic diversity (Araújo et al. 2018).

Carpenter ants infected by *O. unilateralis* have been widely found in tropical forest ecosystems but have been sparsely found in temperate forest ecosystems

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(Araújo et al. 2018). In the last decade, *O. unilateralis* has been documented infecting four native carpenter ant species (*Camponotus castaneus* [Latreille], *Camponotus americanus* Mayr, *Camponotus pennsylvanicus* [De Geer], and *Camponotus floridanus* [Buckley]) in temperate forests in the United States, specifically in South Carolina, Florida, and Louisiana, where infected ants were found to perform their death grip on woody stems rather than leaves (Araújo et al. 2018; de Bekker et al. 2014, 2015; Loreto et al. 2018; Saltamachia and Araújo 2020; Will et al. 2023). Because *Ophiocordyceps* spp. have host-specific tendencies, it is plausible that the genetic diversity and behavioral manipulation strategies of this entomopathogenic fungus could differentiate even further in response to new environments (such as temperate forests). Loreto et al. (2018) showed that fungal development in temperate forest is longer than the period of time leaves are present, which is the likely cause for infected ants being manipulated to bite stems rather than leaves. These stem-biting ants were also found to wrap their legs around the stems, which provided for better attachment to the plant. They also found that the ancestral-state character reconstruction suggests that leaf biting is the ancestral trait and that twig biting is a convergent trait in temperate forests. The findings from Loreto et al. (2018) give further merit to the idea that environmental factors likely play a major role in altering the manipulated behaviors caused by entomopathogenic fungi.

The first known occurrence of this parasitic fungus–ant relationship in the United States was documented in South Carolina in 2009, with collections continuing through 2013 (de Bekker et al. 2014). *Ophiocordyceps unilateralis* exhibits extreme host specificity even among species within the *Camponotus* genus. For example, when de Bekker et al. (2014) inserted fungal cells of *O. unilateralis* into three *Camponotus* spp., only the two ant species known to be naturally associated with *O. unilateralis* (*C. castaneus* and *C. americanus*) exhibited fungal hyphae growth, whereas a similar species not known to be naturally infected as a target host (*C. pennsylvanicus* De Geer) did not (de Bekker et al. 2014, 2015). Only *C. castaneus* and *C. americanus* exhibited the death grip behavior (de Bekker et al. 2014). The relationship between fungal morphologic variation, geographic distribution, and genetic speciation is critical in verifying new occurrences of *O. unilateralis*. In this study we documented the first occurrence of a fungal species within the *Ophiocordyceps* clade (*Ophiocordyceps kimflemingiae*) found in infected *C. americanus* in temperate forests of eastern Tennessee and contributed ecologic observations to better understand this fungus–ant relationship.

Materials and Methods

Site description. This study was conducted at the University of Tennessee (UT) Arboretum (approximately 101 ha) in Oak Ridge, TN (N 35°59′35.934″, W 84° 13′11.7762″). This forested area is a former agricultural site. About 800 species of plants, primarily native to eastern Tennessee, are found at the UT Arboretum. However, some plant species from other parts of the United States and exotic plant species from foreign countries are also found at the UT Arboretum. The UT Arboretum is university owned and operated, and many university forestry trials and experiments are performed there. Because it is open to the public, many residents and local visitors from across the United States use the numerous walking trails.

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Collection information. An infected ant was collected on 22 April 2022 at the UT Arboretum in Oak Ridge, TN. After this initial collection, we began weekly sampling for infected ants at the UT Arboretum from May to December 2022 and from March to May 2023. Five to 10 plant species were inspected weekly during this sampling period (no samples were collected during the winter months) for the presence of infected ants exhibiting the death grip. Because previous studies in the United States documented that infected ants preferred to exhibit the death grip on small woody stems (de Bekker et al. 2017, Saltamachia and Araújo 2020), primarily small ($<$50.0 mm) woody shrubs and tree saplings were monitored for infected ants.

On each sampling date, about 20–25 plants, representing diverse species (such as *Lindera benzoin* (L.) Blume [spicebush], *Ulmus* spp. [elm], *Acer* spp. [maple], and *Betula* spp. [birch]), were visually inspected for approximately 5 min per plant. Ants were classified as infected if they inflicted their mandibular death grip onto the inspected plant host. Plants near (within 5 to 12 m) forest edges were sampled most frequently because of greater accessibility. Leaves, stems, and other plant parts were observed for infected ants.

Infected ants were sampled on plants from ground level up to 2.74 m. However, it is possible that infected ants were present at greater heights on the plants, but we were unable to collect at those heights. Where possible, infected ants were removed by hand. In some cases, removal of intact specimens was not possible, as they fragmented when removed. Specimens that did not remain intact upon removal from the stem were still processed and evaluated. To ensure intact specimens, infected ants within 10 cm of the apical portion of a stem were selected, and approximately 3 cm of this stem around the infected ant were clipped and removed. Seventy stems of various plant species with infected ants that exhibited a death grip were selected, and the stem diameter where the ant was attached, as well as the height of the location of the infected ant, were measured to assess the influence of stem diameter and plant height on attachment by infected ants. The plant species associated with each of the 70 collections were also documented. Once infected ants (both intact and fragmented) were carefully removed by hand, they were placed in plastic bags, transported to the laboratory, and stored in a freezer until further examination.

Fungal morphologic characteristics. Morphologic characteristics of the fruiting body that erupted from the head of the ant were recorded. As mentioned here and in other studies, morphologic characteristics of *O. unilateralis* (*s.l.*) are crucial to understanding the continuously expanding genetic speciation of this fungus. The length (mm) of the fungal stroma and the diameter (mm) of the perithecial plate that is produced laterally on the stroma emerging from the head of the ant were measured and recorded. The number of total ants that were collected also was documented and the understory shrub or tree sapling species from which they were collected was identified. Of the number of specimens collected, the number of ants containing fruiting bodies was calculated.

Deoxyribonucleic acid (DNA) extraction, analysis, and confirmation sequencing. Parts of the stroma (fruiting body of the fungus) were removed from eight infected ants using a new Feather disposable scalpel no. 10 (Feather Safety Razor Co, Osaka, Japan) for each sample. Tissue was disrupted using the TissueLyser II (Qiagen, Hilden, Germany) for two 5-min cycles at 30 Hz. Total DNA was extracted from the fungus using the DNeasy blood and tissue kit (Qiagen)

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according to manufacturer's protocol, with the exception that the elution step was performed twice using 60 μl of buffer AE each time. Extracted DNA was stored in a −20°C freezer until further processing. Molecular confirmation of the morphologic identification was done using primers to amplify the large subunit nuclear ribosomal DNA (LR0R [5′-ACCCGCTGAACTTAAGC-3′] and LR5 [5′-TCCTGAGGGAAACTTCG-3′]) (Araújo et al. 2018, Vilgalys and Sun 1994).

Polymerase chain reaction (PCR) was conducted in 25 μl total volume using 12.5 μl of OneTaq hot-start quick-load 2× master mix with standard buffer (New England Biolabs, Ipswich, MA, USA), 0.25 μM of each primer, 8.5 μl of nuclease-free water, and 2 μl of template DNA. PCR was performed in a Veriti 96-well thermal cycler (Applied Biosystems, Foster City CA, USA). PCR products were visualized with an E-Gel 2% agarose gel (Thermo Fisher Scientific, Waltham, MA, USA) using the E-Gel power snap (Thermo Fisher Scientific).

Samples were cleaned using Exo-CIP rapid PCR cleanup kit (New England Biolabs) to remove excess deoxynucleotide triphosphates, residual single-stranded primers, and DNA. Samples were sent to Eurofins Genomics (Louisville, KY, USA) for bidirectional Sanger sequencing. Resulting sequences were aligned in Genious Prime (GraphPad Software, LLC [d.b.a. Geneious], Boston, MA, USA) and compared with GenBank deposits via the National Center for Biotechnology Information basic local alignment search tool.

Data analysis. Data were analyzed using an analysis of variance. Tukey's honestly significant difference test was used to determine significant differences among the means ($P < 0.05$). All analyses regarding seasonality and fungal morphologic characteristics were performed using R Statistical Software (v4.3.2; R Core Team 2023). All data visualizations were achieved via the tidyverse R package (v1.3.0; Wickham et al. 2019). Significant differences in mean stroma length, mean perithecial diameter, mean stem diameter where the ant was attached to the stem, and mean height of infected ants on host plants among plant species were determined. Significant differences in mean number of specimens collected by month were determined to assess trends in seasonality.

Results

Seasonality. A total of 191 infected ants was collected during this 2-yr study. The mean number of infected ants was statistically different among months (regardless of plant species host from which they were collected) ($F = 6.248$; df $=$ 5, 15; $P = 0.00253$). The mean number of infected ants peaked in September, whereas the lowest mean number was found in December (Fig. 1). Additionally, although almost all infected ants (189) exhibited the mandibular death grip on woody stems (Fig. 2), two specimens exhibited the mandibular death grip on the leaf petiole portion of their plant host. All infected ants that were found biting stems with their legs still attached had them wrapped around those stems (Fig. 2), which agrees with previous findings of infected ant behavior (Loreto et al. 2018).

PCR analysis. Our sequences were >99% similar to *O. kimflemingiae* (GenBank KX713620), a species in the *O. unilateralis* clade (Araújo et al. 2018). The gene sequences have been deposited in GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi) as accession numbers PP503154 and PP503155. The remaining data

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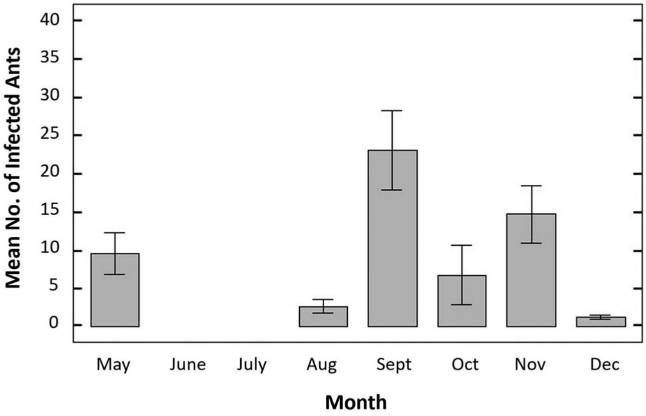

Fig. 1. Mean (+ SE) number of infected ants collected monthly, averaged for 2022 and 2023, Oak Ridge, TN. No infected ants were found in June or July.

used in this manuscript are publicly available within a GitHub repository located at this link: https://github.com/KathleenCoffman/ZAF.

Plant hosts. Infected ants were collected from understory shrubs (such as spicebush) and saplings (including maple, elm, and birch), and a few infected ants were found on a mature *Magnolia macrophylla* Michx. (broadleaf magnolia) during this study (Table 1). Plant species that were singleton or infrequent hosts of infected ants were grouped as "other," which also included plant species that were not identified. Although we have noted plant species from which these specimens were collected, we do not posit any correlation or relationship between infected ant and plant host species from which ant specimens were collected.

Stem diameter and specimen collection height. Stem diameter where infected ants were collected, which averaged 3.39 ± 0.79 mm, was significantly different among plant species ($F = 49.11$; df = 5, 64; $P < 0.00001$) (Table 1). Stem diameter at the attachment site was significantly greater on broadleaf magnolia than on any other plant species. Stem diameter ranged from 0.5 to 45.4 mm; however, the majority (74%) of stems with infected ants ranged from 1.1 to 3.4 mm. Collection

Fig. 2. Mandibular death grip of ants infected with *Ophiocordyceps kimflemingiae* on stems.

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Table 1. Host plants (primarily small woody shrubs and tree saplings) where *Ophiocordyceps kimflemingiae*-infected *Camponotus americanus* were found and corresponding plant measurements, Oak Ridge, TN, 2022–2023.

Plant Species	No. of Infected Ants*	Avg. Stem Diameter (mm)**	Avg. Height of Infected Ant from Ground (m)**
Birch	9 (2)	0.55 ± 0.05a	1.69 ± 0.25ab
Elm	30 (16)	1.55 ± 0.11a	1.20 ± 0.06a
Maple	53 (11)	2.09 ± 0.42a	1.30 ± 0.04a
Spicebush	26 (26)	2.34 ± 0.10a	1.36 ± 0.11a
Broadleaf Magnolia	4 (4)	27.10 ± 6.72b	2.10 ± 0.17b
Other	69 (11)	1.75 ± 0.69a	1.32 ± 0.07a

* Values represent the total number of infected ants collected from each plant species (and the number of plant measurements corresponding to locations of infected ants).

** Means (± SE) within a column followed by the same letter are not significantly different ($P > 0.05$).

height of infected ants, which averaged 1.36 ± 0.05 m above ground level, was significantly different among plant species ($F = 3.711$; df = 5, 64; $P = 0.00518$) (Table 1). Infected ants were found significantly higher in the canopy of broadleaf magnolia than any other plant species except birch (Table 1). Measured heights ranged from 0.13 to 2.52 m; however, collection heights were limited to approximately 2.74 m.

Fungal morphologic characteristics. The average fungal stroma length across all specimens, regardless of their plant host, was more than 6 mm and differed significantly among plant species ($F = 10.83$; df = 4, 108; $P = 2.02\text{e-}07$) (Figs. 3, 4). The average overall perithecial plate diameter was <0.5 mm and did not differ significantly among plant species ($F = 0.633$; df = 4, 43; $P = 0.642$) (Figs. 5, 6). Stroma length and perithecial plate diameter varied greatly among plant species (Figs. 4, 6).

Visible fungal stroma was present on approximately 60% of 191 total specimens. Of those with a fungal stroma present, approximately 44% had a visible perithecial plate. Other specimens (with no visible fungal fruiting body) were still collected as part of this study because they were found attached to woody twigs via the mandibular death grip. As mentioned earlier, ants were classified as infected when observed to exhibit the mandibular death grip on their respective plant host. Some specimens exhibited this distinct mandibular death grip specific to *O. unilateralis* (*s.l.*), but only had partial visible fungal growth (115 with stroma, and of those 115, only 40 had both stroma and perithecial plate). About 18.9% ($n = 36$) of the collected specimens ($n = 191$) had no visible fungal growth present.

Discussion

Although *Ophiocordyceps* spp. have been well documented and studied in the tropical forests of South America, much less is known about these fungi in

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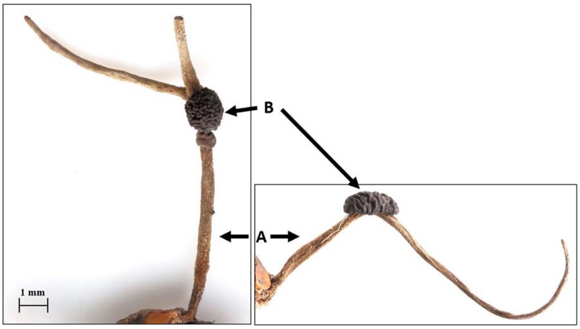

Fig. 3. Fungal stroma (A) and perithecial plate (B) of *Ophiocordyceps kimflemingiae* emerging from the heads of infected *Camponotus americanus*.

the temperate forests of North America. Only a few cases of ZAF have been documented in North America. However, the variation in fungal behavior by the same *Ophiocordyceps* sp. described in this research may imply that this fungus is already more widespread throughout North America than current documentation implies. It is unclear how long the *O. unilateralis* clade has been present in North America. The drastically different traits these fungi exhibit in North American temperate forests (i.e., woody stem attachment) compared with those in South American tropical forests (i.e., leaf blade attachment) imply that they have been present in North America for a sufficient time to adapt differences in how these fungi manipulate ant behaviors to thrive under climate and weather conditions within temperate zones.

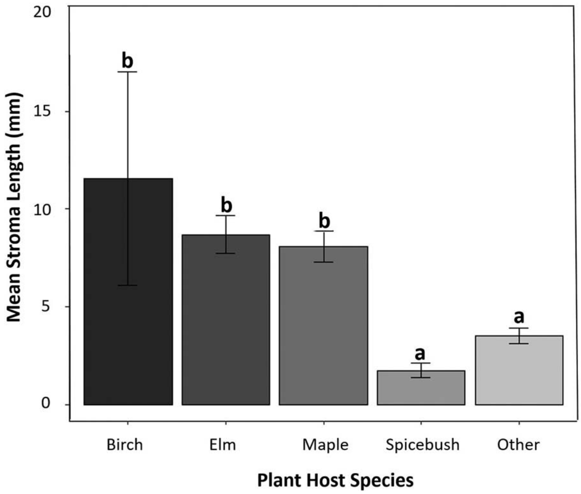

Fig. 4. Mean (± SE) fungal stroma length (mm) of *Ophiocordyceps kimflemingiae* on infected ants collected from various host plant species (average length across all plant species was 6.28 ± 0.40 mm).

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Fig. 5. Close-up of perithecial plate on stroma of *Ophiocordyceps kimflemingiae* collected from an infected *Camponotus americanus*.

Temperate forests in eastern Tennessee, as well as other North American sites where the ZAF has been found, have four distinct seasons including a warm spring, a mild and humid summer, a cool fall, and a cold and dry winter. Their presence in these areas demonstrate that ZAF species can proliferate in North America, which is a climate vastly different from the uniform climate that is present all year in the tropics. Their presence in North America may have caused the behavior manipulation (i.e., death grip attachment) of North American ZAF species. For instance, if infected ants were to inflict their death grip on leaves of temperate woody plant species, where leaves fall from the tree seasonally, the fungus would be less successful during reproduction, thus minimizing spore dispersal. Instead, these fungi alter ant behavior to cause them to bite down on stems rather than leaf blades, which may enable them to remain on the plant through the winter, ensuring fungal reproduction, and infecting new ants in the spring. However, in this study, we did not make observations from January through April.

The diameters of the stems where infected ants were found were relatively consistent. The incidence of infected ants was not dependent upon plant species, as infected ants were found on a diversity of plant species. Some outliers had a slightly smaller or much larger stem diameter. The diameter of stems may correlate with the ability of an ant to perform their death grip, but more research is needed to confirm stem diameter preferences. Other studies showed ants gripping to mats of moss, logs, and even tree trunks (Araújo et al. 2018, Imirzian et al. 2020, Saltamachia and Araújo 2020), so different species of infected ants could exhibit differing death grip behaviors in North America.

Unlike stem diameter, the height at which ant specimens were collected did not have many outliers. Instead, heights of infected ants varied widely. In fact, it is highly possible that ants were also present at much greater heights, even possibly

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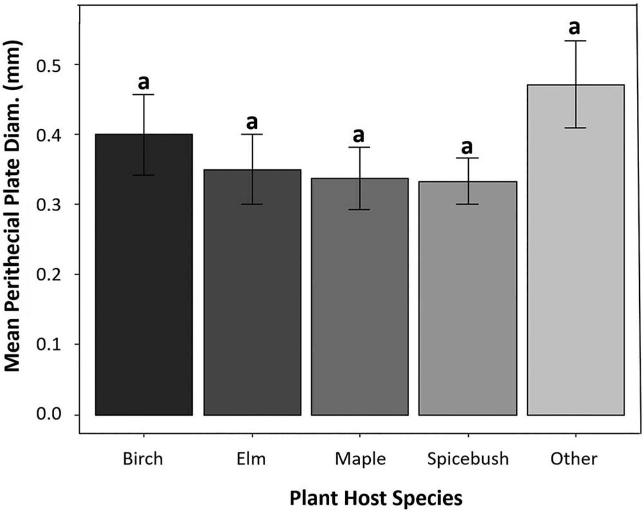

Fig. 6. Mean (± SE) diameter (mm) of perithecial plates on stroma of *Ophiocordyceps kimflemingiae* on infected *Camponotus americanus* collected from various host plant species (average diameter across all plant species was 0.42 ± 0.05 mm) ($P < 0.05$).

in the canopy, but our collection capabilities were limited to a height of about 2.74 m. These results suggest that this species of ZAF present in North America does not cause infected ants to bite down at a specific height, as with infected ants associated with South American species of these fungi (Lin et al. 2020).

As the fungal morphologic data exemplify, *O. unilateralis* fungal fruiting bodies are pleiomorphic, meaning that each one may be morphologically, genetically, and physiologically distinct (Evans et al. 2011b). The perithecial plate is formed on the stroma, and it is a critical aspect of ZAF morphologic characteristics. A vast array of length measurements, ranging from <1 to >30 mm long, were recorded.

The perithecial plate contains the powerful armor of choice for *O. unilateralis*. It houses flask-shaped, thick-walled ascomata that produce saclike structures called asci (Evans et al. 2011a). Each ascus contains sexual spores that are released with pressure, using a slingshotlike mechanism. Our data display perithecial plate morphologic variation, where the diameter measurements range from as small as 0.1 to >1.5 mm wide. Although variations in overall diameter were noticeable, no significant differences in mean diameters were found among plant species ($P = 0.642$).

Conclusions. To our knowledge, these results represent the first confirmed documented case of *O. kimflemingiae* on *C. americanus* in Tennessee. Whereas these collections were similar to other documented cases of ZAF in North America, several differences, even among our collections, were observed. It is possible that the inconsistencies in mandibular death grip location on the plant, plant height, stem diameter, and fungal morphologic characteristics of North American ZAF species are the result of extreme evolutionary changes. These changes contribute to the ability of these

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fungi to conform to the varying climate, weather, plant species, and ant species of temperate forests, which contrast to the consistency of tropical forest systems.

Future research should focus on the phenology of North American ZAF species, host ant specificity, ant behavior manipulation, seasonality trends, and life cycle of the fungi. Because collection height was limited in this study, additional observations at higher levels may inform results to determine if death grip height or location is as important to North American ZAF species as it is to those in South America.

Although ZAF species are generally adapted to manipulate the behavior of only a specific genus or species of ant, it has also been shown that they kill other ant species without causing behavior manipulation (de Bekker et al. 2014). Hypocrealean fungi, such as *Ophiocordyceps*, exhibit high virulence, with mortality rates as high as 100%, because of their ability to overcome arthropod host immune defenses (Chandler 2017). The ability to kill other ant species suggests that ZAF has potential as a natural biologic control for invasive or pest species of ants, such as imported fire ant (*Solenopsis invicta* Buren), odorous house ant (*Tapinoma sessile* [Say]), and Asian needle ant (*Brachyponera chinensis* [Emery]).

ZAF in North America is still a relatively new discovery, but it is likely that it has already spread throughout our temperate forests. Our research provides a foundation for future inquiries into the established timeline of *O. kimflemingiae* in North American temperate forest ecosystems, as well as the evolution of this fungus from its well-established origins in South American tropical forests. As more research studies are conducted with ZAF species, ecologic functioning and biologic control strategies concerning North American ant species in both forest and urban settings will be better understood.

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