Cogongrass (*Imperata cylindrica*) Affects Above- and Belowground Processes in Commercial Loblolly Pine (*Pinus taeda*) Stands

Adam N. Trautwig, Lori G. Eckhardt, Nancy J. Loewenstein, Jason D. Hoeksema, Emily A. Carter, and Ryan L. Nadel

Cogongrass (*Imperata cylindrica*), an invasive grass species native to Asia, has been shown to reduce tree vigor in loblolly pine (*Pinus taeda*) plantations, which comprise more than 50% of growing stock in commercial forests of the United States. *I. cylindrica* produces exudates with possible allelopathic effects that may influence abundance of *P. taeda* symbionts, such as soil microbes and ectomycorrhizal fungi. Soil microbial communities and root colonization by mycorrhizal fungi were sampled in intensively managed *P. taeda* stands in Greene County, Mississippi, in *I. cylindrica* present and absent plots. *I. cylindrica* present plots had reduced abundance of ectomycorrhizal colonization of pine fine feeder roots in the top 40 cm of soil in comparison to *I. cylindrica* absent plots. Abundance of pine fine feeder roots in the 21–40 cm and 41–60 cm layers of the soil profile was also reduced in *I. cylindrica* present plots. Vegetative diversity was negatively correlated with *I. cylindrica* (% cover), which probably contributed to the reduced microbial diversity in *I. cylindrica* present plots. Because of the variety of roles microorganisms play, changes associated with the invasion of *I. cylindrica* are likely to alter nutrient cycling and reduce site productivity.

Keywords: ectomycorrhizal fungi, allelopathy, vegetation

Economic losses attributable to the nearly 50,000 nonindigenous species in the United States are estimated to be US$120 billion per year (Pimentel et al. 2005). A large portion of the impacts of invasive species are ecological in nature and often unnoticed, although they can contribute to ecosystem or community transformation over time (Simberloff et al. 2013). In addition to direct impacts on aboveground flora and fauna, effects on belowground symbionts and other organisms that co-occur with native species can magnify the impact of an invasive species. Although a few instances of compounding effects of coinvasion by plants and their symbionts have been well documented (Richardson et al. 2000, Pasternak et al. 2007, Dickie et al. 2010), principles that outline how an invader will affect preexisting communities of mutualists are generally broader in scope (Ehrenfeld 2003, Wolfe and Klironomos 2005, van der Heijden et al. 2008).

The role of diversity in understory vegetation has been thoroughly explored (Nilsson and Wardle 2005, Gilliam 2007, Hector and Bagchi 2007). Monoculture-forming invaders that replace entire communities of native plants have the potential to affect microorganism communities and canopy vegetation (Callaway and Ridenour 2004, Stinson et al. 2006). *Imperata cylindrica* invasion of a longleaf pine (*Pinus palustris* Mill.) ecosystem resulted in a loss of the herbaceous plants that make this native ecosystem distinct (Brewer 2008). The loss of these herbaceous species may have significant effects on threatened ecosystems (Brewer 2008). These significant effects have been well established in the literature and include alteration of fire regimes, degradation of habitat, and altered nutrient cycles (Lippincott 2000, Daneshgar and Jose 2009, Hagan et al. 2013b) Cogongrass (*Imperata cylindrica* [L.] Beauv) is classified as a noxious weed in more than 70 countries and represents a tangible threat to biodiversity and resource management (Burrell et al. 2015, Estrada and Flory 2015). In areas where it has successfully invaded, it often forms dense monotypic stands (MacDonald 2004). *I. cylindrica* was introduced accidentally from Japan to the United States in 1912 but later was intentionally introduced for forage crop trials and erosion control (Bryson and Carter 1993, Holzmueller and Jose

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I. cylindrica is a C₄, rhizome-producing, perennial plant that can reach heights of 3 m but typically grows to heights of 1.2 m (Koger and Bryson 2004). It spreads both sexually through seeds and asexually through rhizomes. Seed production can be prolific with most seeds generally dispersed within 15 m of the plant (Daneshgar et al. 2008). I. cylindrica can grow on a variety of soils, from nutrient-poor, coarse sands to nutrient-rich, sandy loam soils (Jose et al. 2002). It is particularly difficult to eradicate because it produces a vast network of underground rhizomes from which the plant regenerates. Rhizomes can reach densities of 89 m⁻² of underground rhizomes from which the plant regenerates. Rhizomes spread both sexually through seeds and asexually through rhizomes. Seed production can be prolific with most seeds generally dispersed within 15 m of the plant (Daneshgar et al. 2008). I. cylindrica can grow on a variety of soils, from nutrient-poor, coarse sands to nutrient-rich, sandy loam soils (Jose et al. 2002). It is particularly difficult to eradicate because it produces a vast network of underground rhizomes from which the plant regenerates. Rhizomes can reach densities of 89 m⁻² of soil (MacDonald 2004).

Daneshgar and Jose (2009) found that competition for nitrogen (N) between I. cylindrica and loblolly pine (Pinus taeda L.) seedlings resulted in a greater reduction in pine seedling growth compared to competition with native vegetation. I. cylindrica also may impact growth of timber species such as P. taeda through production of exudates that may have an allelopathic effect (Hussain and Abidi 1991, Koger and Bryson 2004, Holzmueller and Jose 2011, Hagan et al. 2013b). Compounds isolated from exudates of I. cylindrica with possible allelopathic effects include seven phenolic acids, two aromatic acids, one trihydroxy anthraquinone, and one meta-dihydroxy phenol (Hagan et al. 2013b). In addition to direct impacts on plant growth, compounds present in I. cylindrica exudates may affect growth through influence on mycorrhizae and other soil microbial taxa (Roberts and Anderson 2001, Kourtev et al. 2002, Hagan et al. 2013b). Boufalais et al. (1994) found that the growth of two ectomycorrhizal fungal species, Cenococcum geophilum and Laccaria lacaca, were affected by the applications of several phenolic acids similar to those produced by I. cylindrica. For this study, we hypothesized that I. cylindrica present P. taeda plots would have reduced mycorrhizal and fine P. taeda feeder root abundance, a reduction in microbial biomass (N) (mg · liter⁻¹) and P. taeda diameter growth rate (mm · year⁻¹), and reduced vegetation diversity compared to I. cylindrica absent plots.

**Materials and Methods**

**Site Description**

The research site was located East of Leakesville, in Greene County, Mississippi, an area under commercial P. taeda production (31.148493, −88.470755). The area receives an average annual rainfall of 1,677 mm. The dominant mid-story vegetation includes yaupon (Ilex vomitoria Ait.), scrub oak (Quercus spp.), and P. taeda. Much of the area has also been invaded by I. cylindrica. The soil series was identified as Benndale sandy loam on 8–15% slopes and Benndale sandy loam or McLaurin sandy loam on 2–5% slopes. Benndale soils are classified as a coarse-loamy, siliceous, semiactive, thermic Typic Paleudults, and McLaurin soils are classified as a coarse-loamy, siliceous, subactive, thermic Typic Paleudults. These soils are well drained and generally nutrient-poor due to low amounts of organic matter and clay (Soil Survey Report, Greene County, 2012).

A total of eight study plots (16 m x 16 m) were established within two similar stands of P. taeda, which have received slightly different management regimens (Table 1).² The plots were situated within the northeast quadrant of larger plots (30 m x 30 m), which were initially established for a study in 2010 (Brunson 2013). I. cylindrica was present at this site before the establishment of that study in 2010, but the exact time of invasion is not known. Four plots were located within areas of the stands that were not invaded with I. cylindrica; the remaining four were in areas that were invaded with I. cylindrica. Over the course of this study, I. cylindrica invaded one of the non-I. cylindrica plots, and it was determined that I. cylindrica density in one of the I. cylindrica-invaded plots was lower than in the original larger plot as a whole. Therefore, for analyses, the plots were characterized by whether I. cylindrica was present (<50% cover, n = 2), abundant (>50% cover, n = 3), or absent (n = 3). Sites were all in close proximity and represent a high degree of homogeneity with the exception of I. cylindrica being present.

**Mycorrhizae**

Field sampling of roots for mycorrhizae was performed in November 2014 and May 2015. Preliminary studies have shown that May is the most reliable time to survey mycorrhizal communities as this is when mycorrhizal fungi are most active in this region (J. Hoeksema, University of Mississippi, pers. obs., Aug. 4, 2013). Another sampling in November was included to account for seasonal variation (Brundrett et al. 1996). At each plot, nine evenly distributed soil cores (10-cm diameter x 60-cm height) were removed across each of the eight plots using a pneumatic coring device. This method of soil core sampling assured that both the O and A soil horizons were sampled for mycorrhizae. Although the majority of I. cylindrica rhizomes were found higher in the soil profile, roots have been found as deep as 120 cm (MacDonald 2004). Therefore, an analysis of multiple layers of soil was necessary. Each soil core was taken approximately 2.5 m from the next core, and each plot contained several trees. Individual cores were sealed and immediately stored at 4°C until processing.

Soil cores were cut into 20-cm increments, and roots were gently washed free of soil with water through a 0.5-mm sieve (Horton and Bruns 1998, Roberts and Anderson 2001). Roots were pooled within each plot by soil core increment (0–20, 21–40, and 41–60 cm). Pine roots were separated and kept in a 2% cetyl trimethyl ammonium bromide (CTAB) lysis buffer (Tedersoo et al. 2006). Because ectomycorrhizal tips were abundant (7–72 x 10⁷ · m⁻²) (Taylor 2002), we arbitrarily sampled 100 1-cm root segments for a total of 100 cm of fine roots per 20-cm soil core increment except when there were not enough roots present, in which case we examined all roots (Anderson et al. 2010), to measure the percentage of fine root segments that were colonized. The lengths of fine feeder roots (<2 mm) were measured (cm) for all plots. Mycorrhizal structures on subsampled roots were identified and quantified at X10–40 magnification using the gridline intercept method (Brundrett et al. 1996).

**Vegetation Survey**

A vegetation analysis was undertaken in July 2014 to quantify percent cover of vegetation on I. cylindrica-invaded and noninvaded plots. Vegetation was assessed using a 10% scale by the line transect

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**Table 1. Site conditions and treatments listed by plot.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>I. cylindrica absent</th>
<th>I. cylindrica present (&lt;50% cover)</th>
<th>I. cylindrica abundant (&gt;50% cover)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. cylindrica cover 2011 (%)</td>
<td>0 (0)</td>
<td>43.00 (43.00)</td>
<td>85.00 (10.54)</td>
</tr>
<tr>
<td>Stand age</td>
<td>18.00 (2.00)</td>
<td>21.00 (0)</td>
<td>18.00 (2.00)</td>
</tr>
<tr>
<td>Basal area (m²/ha)</td>
<td>22.43 (1.20)</td>
<td>25.30 (0)</td>
<td>22.43 (1.20)</td>
</tr>
<tr>
<td>Trees per acre</td>
<td>245.67 (11.29)</td>
<td>243.00 (0)</td>
<td>245.67 (11.29)</td>
</tr>
<tr>
<td>Percent stocking</td>
<td>73.33 (3.33)</td>
<td>80.00 (0)</td>
<td>73.33 (3.33)</td>
</tr>
</tbody>
</table>

Plots were divided into no I. cylindrica observed, plots with <50% I. cylindrica and plots in which I. cylindrica was present at >50%. Average values are displayed with standard errors in parentheses.
method, where 10 adjacent 1-m² quadrats were sampled on a diagonal transect within each plot (Krebs 1989). Plants that were present but in lower abundance than 10% were given a classification of 5% to ensure they were accounted for in diversity estimates. Vegetation that could not be identified in the field was collected and pressed for later identification by plant taxonomists at Auburn University.

**Microbial Biomass N**

Four soil samples were collected from each plot to determine organic N levels (mg/liter), which were used to calculate microbial biomass N. Samples were collected 2 m apart in each cardinal direction, in the southwest corner of each plot or the center of the original plot. Before sampling, the top layer of loose organic debris (or duff) was removed. Because target microbial communities are more abundant higher in the soil profile, only the upper layer of soil (≤10 cm) was sampled. Samples were placed in plastic bags and transported within 8 hours to the laboratory where they were refrigerated. All tests were performed within 3 days.

Soil samples were sieved using a 2-mm sieve. Each soil sample was separated into two 18.5-g samples and was processed using the chloroform-fumigation extraction technique (Vance et al. 1987). This method measures only the organic N in microbial organisms in the soil by lysing cell walls in one sample (using chloroform) and not disturbing the second sample. Organic N levels were measured utilizing a TNM-1 total N measuring unit (Shimadzo Scientific Instruments, Columbia, MD). The organic N levels from the nondisturbed samples are subtracted from the organic N levels in the fumigated sample. The subtracted value, divided by the efficiency of extraction constant, yielded the microbial biomass N. These values for the four soil samples collected per plot were averaged before plot comparisons.

**Tree Radial Growth**

Within each of the original 30-m × 30-m plots, six dominant or codominant trees closest to plot center were selected to evaluate tree growth rate. An increment borer was used to remove a tree core at breast height (1.37 m). Cores were mounted onto blocks and sanded down to show clear annual growth rings. Total radial growth over the last 5 and 10 years was determined by measuring from the early wood of the 5th and 10th annual rings, respectively, to the bark. To account for differences in growth due to relative density of trees on each plot, growth was expressed as a function of percent stocking. Percent stocking standardizes available growing space across plots with trees of various ages and sizes and differing basal area (Gingrich 1967).

**Statistical Analysis**

Analyses examining mean percent colonization and mean fine root length as response variables were conducted in R version 3.0.2, using the lmerTest package. Graphs were constructed in STATISTICA (2013; StatSoft, Tulsa, OK). Mean values were calculated from multiple cores (nine) in each plot on each sampling date. We performed split-plot, repeated-measures mixed-model analyses of variance (ANOVCs) in which *I. cylindrica* abundance was a whole-plot between-subjects factor, sampling depth was a split-plot between-subjects factor, sampling date was a within-subjects factor, and plot was a random factor. These models included treatment, depth, month, and all two-way interactions between them as fixed factors. The three-way interaction among all three was excluded from final models, as it was always highly nonsignificant.

Models were fit with the lmer function using restricted maximum likelihood. Degrees of freedom and *P* values were estimated using the Satterthwaite method. Marginal means and standard errors for significant predictors were obtained using the lsmeans function, and pairwise significant differences between means were estimated using the diffmeans function. For significant interactions of treatment with depth or month, we only conducted pairwise tests between levels of treatment within each depth or month to minimize the number of pairwise tests conducted. Differences were considered statistically significant at *α* = 0.05.

SAS version 9.3 (2010; SAS Institute, Inc., Cary, NC) and STATISTICA were used for one-way ANOVA on microbial biomass with *I. cylindrica* invasion as the predictor variable. In addition, a factorial ANOVA examining the effect of *I. cylindrica* presence and loblolly stocking percentage was conducted on radial growth measurements. A post hoc Tukey honestly significant difference test (*α* = 0.05) was performed to compare means in pairwise tests. Residuals were tested for normality and response variables were log transformed when found to be nonnormally distributed.

Species richness and diversity for each plot were calculated using both the Shannon-Wiener and Simpson’s nonparametric measurements of diversity (Colwell 2013, Magurran 2013). A Student’s *t*-test was undertaken to compare mean richness and diversity across invasion category. Percent cover of *I. cylindrica* was regressed against both the Shannon-Wiener and Simpson’s diversity indices to determine whether the two were correlated.

**Results**

**Mycorrhizae**

Mycorrhizal fungi colonization was affected by a significant interaction between *I. cylindrica* abundance and month [*F*(_2_, _29_) = 5.53, *P* = 0.009] (Figure 1). *I. cylindrica* exercised a different influence on mycorrhizal fungi colonization in November than in May. Overall, there is a trend toward decreased colonization by mycorrhizal fungi in the presence of *I. cylindrica*, but in November mycorrhizal colonization was significantly lower in *I. cylindrica* present plots than in *I. cylindrica* abundant plots. In May, the comparison of mycorrhizal colonization in plots with no *I. cylindrica* versus *I. cylindrica*...
present plots was nearly significant ($P < 0.1$), despite very low replication ($n = 3$ plots each). Mycorrhizal fungi colonization was also significantly different across depth classes (Figure 2). Specifically, fine roots at depths of 0–20 cm were more heavily colonized than those at depths of 21–40 cm ($P < 0.0001$) or 41–60 cm ($P < 0.0001$). Similarly, roots in the 21–40 cm depth class were more heavily colonized than those in the 41–60 cm depth class ($P < 0.0001$).

Amount of fine roots recovered was significantly affected by an I. cylindrica abundance × month interaction. In both November and May, I. cylindrica absent plots yielded significantly more P. taeda fine roots than I. cylindrica present ($P = 0.038$ and $P < 0.0001$) and I. cylindrica abundant plots ($P < 0.0001$ and $P = 0.005$) (Figure 3). Fine root abundance was also affected by a significant interaction between depth class and I. cylindrica abundance. In the 21–40 cm depth class I. cylindrica absent plots yielded more fine roots than either I. cylindrica present or abundant plots ($P < 0.0001$ and $P < 0.0001$), respectively). Similarly, in the 41–60 cm depth class I. cylindrica absent plots yielded more fine roots than I. cylindrica present or abundant plots ($P = 0.008$ and $P < 0.0001$, respectively) (Figure 4).

Table 2. Percent cover of Imperata cylindrica and bare ground on plots, as well as species richness and measures of diversity.

<table>
<thead>
<tr>
<th></th>
<th>I. cylindrica absent</th>
<th>I. cylindrica present</th>
<th>I. cylindrica abundant</th>
</tr>
</thead>
<tbody>
<tr>
<td>2015 (%)</td>
<td>0 (0)</td>
<td>17.00 (5.00)</td>
<td>80.67 (13.91)</td>
</tr>
<tr>
<td>Bare ground (%)</td>
<td>35.33 (12.72)</td>
<td>32.00 (4.00)</td>
<td>9.00 (6.51)</td>
</tr>
<tr>
<td>Species richness</td>
<td>13.33 (2.60)</td>
<td>11.50 (0.50)</td>
<td>5.33 (1.67)</td>
</tr>
<tr>
<td>Shannon-Wiener index</td>
<td>1.77 (0.32)</td>
<td>1.81 (0.03)</td>
<td>0.49 (0.29)</td>
</tr>
<tr>
<td>Simpson index</td>
<td>4.49 (1.54)</td>
<td>4.47 (0.03)</td>
<td>1.39 (0.30)</td>
</tr>
</tbody>
</table>

Virtual values are displayed with standard errors in parentheses.

Vegetation Survey

Plant species richness and diversity differed between the I. cylindrica abundant and I. cylindrica absent plots for both Shannon-Wiener ($P = 0.049$) and Fisher’s $\alpha$ ($P = 0.042$) indices but not Simpson’s diversity index ($P = 0.120$) (Table 2).

Individual species and both observed diversity indices were correlated for each species. I. cylindrica was the only species significantly negatively correlated with measures of diversity. An inverse correlation was also observed in a linear regression between I. cylindrica percent cover and Shannon-Wiener diversity index ($y = 1.8926 – 0.0172x, r = 0.9027, P = 0.0021, r^2 = 0.815$) (Figure 5A) as well as I. cylindrica and Simpson’s diversity index ($y = 4.6486 – 0.0385x, r = 0.7299, P = 0.0398, r^2 = 0.533$) (Figure 5B).

Microbial Biomass N

A significant interaction was not observed between I. cylindrica and month of sampling. Furthermore, no significant differences were observed in either I. cylindrica abundance or time on microbial biomass N. There is a consensus in the literature that microbial biomass is related to soil moisture (Wardle 1992, Devi and Yadava 2006). A related study on the same site did not find a relationship
between microbial biomass N and soil moisture \( y = 4.8233 + 0.1803x; r = 0.2919; P = 0.1050; r^2 = 0.0852 \) (Trautwig 2015).

**Radial Growth**

No significant differences in radial growth were observed between *I. cylindrica* present and absent plots. No *I. cylindrica* present plots were located in stands with lower than 70% stocking (Table 1). Plots in stands with lower stocking densities tended to have higher 5- and 10-year growth rates than higher stocked stands.

**Discussion**

Mycorrhizae are responsible for many plant functions, in particular nutrient uptake. There is significant consensus within the published literature concerning the role mycorrhizal fungi play in contributing to nutrient uptake, particularly in the case of immobile nutrients (Bolan 1991, Marschner and Dell 1994, Landeweert et al. 2001). With a significant reduction in colonization by ectomycorrhizal fungi, as observed here in *P. taeda* plots with high abundance of *I. cylindrica* during the November sampling (Figure 1), the volume of soil the plant is able to exploit is reduced, potentially resulting in reduced uptake of essential nutrients. It is important to note that ectomycorrhizal fungi predominantly colonize a host’s fine root tips, more abundant in topsoil (Brundrett et al. 1996). Although the magnitude of the reduction in mycorrhizal colonization density (percent colonization of fine feeder roots) is not dramatic, the reduced abundance of fine feeder roots also observed on the invaded sites, especially deeper in the soil profile and in the November field sampling period (Figures 3 and 4), probably compounds the effect of reduced mycorrhizal colonization in more heavily invaded sites, and hampers the ability of trees to take up nutrients directly. Although significant differences in mycorrhizal colonization were only noted between *I. cylindrica* present and *I. cylindrica* abundant in November, we noted a trend toward increasing cover of *I. cylindrica* leading to decreased mycorrhizal colonization in both field seasons. In particular, the *I. cylindrica* absent and *I. cylindrica* abundant plots showed a nearly significant difference \( P = 0.066 \), despite very low replication \( (n = 3) \). Similar studies have found that *I. cylindrica* reduced belowground root biomass in *P. taeda* seedling and

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**Figure 5.** Linear regression of Shannon-Weiner index and *I. cylindrica* percent cover \( (n = 8) \) (A) and Simpson diversity index and *I. cylindrica* percent cover \( (n = 8) \) (B).
percent colonization of mycorrhizae in slash pine (*Pinus elliottii* Engelm.) seedlings (Daneshgar et al. 2008, Hagan et al. 2013b). This study supports those trends in an established pine forest environment. We theorize that the differences observed in mycorrhizal abundance and fine root abundance were predominantly attributable to the thick rhizomorphic mats that *I. cylindrica* forms and the potentially allelopathic compounds *I. cylindrica* produces (Hagan et al. 2013b). Several studies have postulated that potentially allelopathic chemicals produced by *I. cylindrica* may play a role in its invasive success (Koger and Bryson 2004, Holzmuller and Jose 2011, Hagan et al. 2013b).

We found that there was no significant difference in plant diversity indices between *I. cylindrica* present, abundant, and absent plots. However, when *I. cylindrica* abundant and *I. cylindrica* absent plots were compared, there was a significant difference. We also found that *I. cylindrica* percent cover was negatively related to vegetation diversity. Decreased plant diversity associated with invading vegetation is not a novel observation (Breuer 2008, Vilá et al. 2011). The effect of such a homogenization of plant diversity on ecology at the landscape scale may be severe (Chapin et al. 2000). It is well documented that diverse plant communities are more microbially active (Kowalchuk et al. 2002, Zak et al. 2003, Eisenhauer et al. 2010). Microorganisms play an imperative role in nutrient cycling, in particular through mineralization and competition for nutrients (van der Heijden et al. 2008). Because dense monocultures of *I. cylindrica* reduce plant diversity, microorganisms associated with extirpated plants, as well as nonassociated beneficial microorganisms, are probably being extirpated simultaneously, disrupting nutrient cycling. Counter to these expectations Hagan et al. (2013a) found that *I. cylindrica* did not have significant direct results on nutrient dynamics although eradication produces significant results with respect to phosphorus (P) and N. Specifically, no differences in nutrient dynamics although eradication produces significant results found that *I. cylindrica* may play a role in its invasive success (Koger and Bryson 2004, Holzmuller and Jose 2011, Hagan et al. 2013b).

**Conclusion**

Abundances of mycorrhizal fungi and fine feeder roots were significantly reduced in *I. cylindrica* abundant plots. Vegetative communities were also less diverse in *I. cylindrica* abundant plots. These factors probably contribute to a lower overall microbial diversity and a reduction in the rate of nutrient cycling and affect the soil nutrients present. Although impacts on growth were not observed, it is expected that over time, an impact may be seen. Furthermore, these changes may lead to communities that are more susceptible to disease and secondary invasion. Careful monitoring and *I. cylindrica* control may be necessary to maintain production of *P. taeda* and conservation of biodiversity.

**Endnotes**

1. For more information, see www.idcide.com.
2. The 16-year-old stand was thinned in 2009 and has not been burned. Diammonium phosphate (DAP) was applied in 2006 at a rate of 280 kg/ha, and urea was applied in 2010 at a rate of 224 kg/ha. The 21/22-year-old stand was thinned before 2006 and had a cool season controlled burn in 2009. It also had DAP applied in 2006 but at a rate of 140 kg/ha and urea was applied in 2007 at a rate of 224 kg/ha.

**Literature Cited**


Colwell R.K. 2013. EstimateS: Statistical estimation of species richness and


