

Effect of the Systemic Neonicotinoid Insecticide Dinotefuran on Leaf-level Photosynthesis in Loblolly Pine (*Pinus taeda*)

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ABSTRACT

Bark beetles (Coleoptera: Curculionidae: Scolytinae) are a common pest in loblolly pine (*Pinus taeda* L.) plantations, and climate intensification threatens to increase the range of bark beetle infestation and vulnerability of loblolly pine. New insecticides and management options are needed to maintain loblolly pine plantation productivity, mitigating increased bark beetle risk. The systemic neonicotinoid insecticide dinotefuran (Safari 20SG systemic insecticide, Valent USA, LLC), is utilized by nursery managers to protect loblolly pine from bark beetles, but there is little published literature on the insecticide's potential phytotoxicity. The objective of this study was to evaluate the effect of dinotefuran on the leaf-level photosynthesis of three loblolly pine genotypes. Dinotefuran was applied to lower tree boles, and photosynthetic measurements occurred two months later. Dinotefuran did not significantly impact leaf-level gross photosynthesis (P_{gmax}), light-saturated net photosynthesis (P_{net}) or dark respiration (R_d); nor was there any significant genotypic variation in responses. Dinotefuran appears to be a promising insecticide for bark beetle prevention without risk of foliar toxicity.

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Introduction

Loblolly pine is the second-most common tree in the continental U.S. and the principal timber species of the Southeast (Oswalt et al. 2019). Current climate projections predict that the United States will experience climate intensification over the coming century as temperatures increase and precipitation patterns change (Melillo, Richmond, and Yohe 2014). Increasing temperatures and more sporadic precipitation have the capacity to increase tree drought stress (McNulty et al. 2019), and current research suggests that forests are already experiencing more stressful climate conditions compared to historic levels (Ficklin and Novick 2017).

Major insect pests of loblolly pine include bark beetles (Coleoptera: Curculionidae: Scolytinae), which encompass several insect pests such as southern pine beetle (*Dendroctonus frontalis* Zimmerman) and *Ips* species (Schowalter 2012). Bark beetles colonize the phloem of southern pines where they can damage vascular tissue and introduce fungal pathogens (Schowalter 2012). The most economically destructive pest to *Pinus* in the Southeast is southern pine beetle that causes about \$43 million dollars in economic losses per year (Pye et al. 2011). Southern pine beetle is endemic to southeastern states, with its historic northern extent around the southern tip of New Jersey (Williams and Liebhold 2002). The northern range limit of southern pine beetle is restricted by winter minimum temperatures (Duehl, Koch, and Hain 2011), and a warmer climate may permit northern range expansion and an increase in the number of beetle generations per year (Ungerer, Ayres, and

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Lobardero 1999; Williams and Liebhold 2002). In the northern part of the pest's range, an unusually severe southern pine beetle outbreak caused more than 90% mortality in pitch pine (*Pinus rigida* Mill.) forests located in the Wertheim National Wildlife Refuge, Long Island, New York (Dodds et al. 2018). The risk of southern pine beetle outbreaks increases following stressful weather events that cause tree injury (Schowalter 2012). Drought may also increase southern pine susceptibility to bark beetle attacks (Huang et al. 2019; Schowalter 2012), with one experimental study finding that water deficits increased the likelihood of successful bark beetle colonization in loblolly pine (Dunn and Lorio 1993).

Bark beetles are typically controlled through management techniques such as thinning or application of insecticides (Schowalter 2012). Registered insecticides for southern pine beetle include the broad-spectrum insecticides bifenthrin and permethrin. However, application of these insecticides can be challenging due to the need to soak the entire stem which risks fish and non-target insect death by drift spray (Billings 2011). Systemic insecticides offer a promising alternative to traditional insecticides, because systemic chemicals can be applied to the soil where they are absorbed by tree root systems and transported throughout the tree by vascular tissues (Billings 2011). This system of action reduces the risk of drift spray, increases the ease of application, and may increase the duration of pest protection since systemic insecticides are incorporated into tree tissues (Billings 2011).

Dinotefuran (Safari 20SG; Valent USA, LLC, Walnut Creek, California) is a systemic neonicotinoid insecticide registered for the protection of ornamental trees and vegetable crops against pests such as mountain pine beetle (*Dendroctonus ponderosae* Hopkins) and emerald ash borer (*Agrilus planipennis* Fairmaire) (Valent USA LLC 2015). Dinotefuran can be applied either to bark or soil where it is absorbed into the vascular system and translocated throughout the tree (Valent USA LLC 2015). McCullough et al. (2011) reported that dinotefuran applied to the bole of ash trees (*Fraxinus* spp.) successfully reduced emerald ash borer colonization. Also, foliage of treated trees was toxic to emerald ash borer due to the presence of dinotefuran (McCullough et al. 2011).

Little published literature has evaluated the efficacy of dinotefuran for bark beetle prevention in southern pines, but some seed orchard managers utilize dinotefuran to protect loblolly pine from bark beetles (Dr. Michael Blazier, personal conversation with orchard managers, April 15, 2019). A preliminary study of systemic insecticides found that emamectin benzoate outperformed dinotefuran at protecting loblolly pine from *Ips* species, but emamectin benzoate caused significant wood deformations that were not observed in dinotefuran-treated trees (Grosman and Upton 2006). Phytotoxicity, in part due to interference in photosynthetic processes, has been reported with the use of other systemic neonicotinoid insecticides (Shakir et al. 2018, 2016). For example, imidacloprid application decreased the concentration of photosynthetic chlorophyll pigments when applied at or below the recommended dose (Shakir et al. 2016). Todorenko et al. (2020) reported that the systemic neonicotinoid insecticide thiamethoxam interfered with leaf-level photosynthesis and that sensitivity to the insecticide varied by genotype. The presence of dinotefuran in leaf tissue was detected even 14 months after application (McCullough et al. 2011). While this suggests that dinotefuran provides long-term protection, the post-application duration of dinotefuran in leaf-tissue could interfere in leaf physiology and subsequently, tree productivity and health. The objective of this study is to evaluate the effect of dinotefuran on leaf-level physiology in loblolly pine and to compare genotypic responses to the insecticide by examining maximum gross photosynthesis (P_{gmax}), light-saturated net photosynthesis (P_{net}) and dark respiration (R_d).

Materials and methods

Study site and experimental design

Dinotefuran evaluation was conducted in a 15-year-old loblolly pine plantation at the Louisiana State University Agricultural Center Hill Farm Research Station (32.749025 N, -93.04111111 W). Trees were planted in 2004 with several genotypes, three of which were utilized in this study: 1) a genetically improved open-pollinated Louisiana genotype (LA), 2) a genetically improved open-pollinated

Carolina coastal plain genotype that is commonly planted through the southeast (756), and 3) a clonal variety propagated from rooted cuttings of a robust 756 individual (93). Additional site details, soil characteristics, and genotype information can be found in Blazier et al. (2018).

On June 11, 2019, dinotefuran (Safari 20SG systemic insecticide, Valent USA, LLC, Walnut Creek, CA, USA) was applied during a dry period in accordance with Safari label directions. Insecticide solution was mixed at a ratio of 94 ml Safari: 1 L water. Insecticide application occurred with an ATV-mounted sprayer to the lower 1 m of tree boles. Each side of the tree was soaked to ensure insecticide uptake, and in total each tree received 502 ml of Safari solution (18 g active Safari per cm of trunk) over a 15 second period.

A completely random and balanced factorial experimental design was utilized with six treatment combinations representing three genotypes (LA, 93, 756) and two insecticide treatment levels (insecticide, control) replicated four times for a total of 24 experimental units (trees). Trees included in the study were selected to minimize diameter (average 21.1 cm, $\sigma = 0.8$ cm) and height differences (average 18.3 m, $\sigma = 1.4$ m), but some inherent height variation between genotypes was unavoidable (Blazier et al. 2018).

Leaf-level photosynthesis

Leaf-level physiology was evaluated with a LI-6400XT portable photosynthesis system (LI-COR Biosciences, Lincoln, NE, USA) with an infrared gas analyzer (IRGA) 6-cm² cuvette equipped with a blue red LED light source. Measurements were conducted 2 months after insecticide application during two consecutive sunny days, August 21–22 2019. Foliage from the upper one-third of the canopy was accessed using a shotgun to extract branches, and branch ends were immediately wrapped in Parafilm (Bemis Company Inc., Neenah, WI, USA) after excision. Two fascicles from the first fully elongated flush of 2019 were clamped inside the IRGA cuvette to measure photosynthesis. The IRGA cuvette environment was controlled such that CO₂ was maintained at 405 $\mu\text{mol mol}^{-1}$, fan speed was set to high, and flow rate was maintained at 300 $\mu\text{mol s}^{-1}$, chamber humidity was not scrubbed, and light intensity was set to simulate light and dark photosynthesis reactions. For each measurement, photosynthetic photon flux densities in sequence were 2000, 1500, 1000, 800, 600, 400, 200, 100, and 0 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Following photosynthetic measurements, fascicle diameter was measured with calipers and number of needles per fascicle was recorded. Fascicle dimensions were used to adjust photosynthesis values by needle surface area using the method from Ginn et al. (1991).

$$A = 2RL(N + \pi) \quad (1)$$

where A is foliage surface area (cm²), R is fascicle radius (cm), L is the length of the IRGA cuvette (3 cm), and N is the number of needles per fascicle. After photosynthetic measurements were adjusted by needle surface area, light response variables were derived using light-curve fitting software (Lobo et al. 2013). Based on the recommendations of Lobo et al. (2013), nine models were evaluated and the model with the lowest sum of squares error was selected. The model that performed best was the Kaipiainen (2009) rectangular hyperbola Michaelis-Menten-based model,

$$P_{net} = \frac{I \times P_{gmax}}{I + I_{(50)}} - R_d \quad (2)$$

where P_{net} is light-saturated net photosynthesis [$\mu\text{mol}(\text{CO}_2) \text{ m}^{-2} \text{ s}^{-1}$], I is light intensity [$\mu\text{mol}(\text{photon}) \text{ m}^{-2} \text{ s}^{-1}$], P_{gmax} is maximum gross photosynthesis, [$\mu\text{mol}(\text{CO}_2) \text{ m}^{-2} \text{ s}^{-1}$], $I_{(50)}$ is the light intensity where P_{net} is 50% of P_{gmax} [$\mu\text{mol}(\text{photon}) \text{ m}^{-2} \text{ s}^{-1}$], and R_d is dark respiration [$\mu\text{mol}(\text{CO}_2) \text{ m}^{-2} \text{ s}^{-1}$].

Table 1. Probabilities of a greater F-value for two-way analyses of variance of loblolly pine leaf-level photosynthetic variables in response to genotype, insecticide treatment, and their interaction.

Fixed Effects	P_{gmax}	P_{net}	R_d
Genotype	0.2012 (2)	0.2221 (2)	0.2052 (2)
Insecticide	0.8858 (1)	0.9928 (1)	0.5592 (1)
Genotype x Insecticide	0.6421 (2)	0.7313 (2)	0.5385 (2)

^a P -values and degrees of freedom (parentheses). Fixed effects were considered significant at $\alpha \leq 0.10$

Statistical analysis

The effect of dinotefuran on leaf-level physiology was evaluated by two-way analyses of variance (ANOVA) conducted at $\alpha = 0.10$. Fixed effects were considered significant at the alpha level of 0.10 to account for the inherent environmental variation within large tree canopies (Tang et al. 1999). The ANOVA analyses utilized a generalized linear model using PROC GLM in SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Insecticide treatment (insecticide, control) and genotype (LA, 93, 756) were incorporated into the model as fixed effects to evaluate main and interaction effects on maximum gross photosynthesis (P_{gmax}), light saturated net photosynthesis (P_{net}), and dark respiration (R_d). Significant main and interaction effects were further analyzed using LSMEANS to conduct Fisher's least significant differences means separation test.

Results and discussion

Photosynthesis and dark respiration play a major role in pine carbon metabolism (Ryan et al. 1994). Our data analyses found that application of dinotefuran did not have a statistically significant effect on leaf-level P_{gmax} ($P = .89$), P_{net} ($P = .99$), or R_d ($P = .55$) (Table 1). Despite the presence of dinotefuran in leaf tissue (McCullough et al. 2011), dinotefuran application did not interfere with leaf-level carbon metabolism. The ability of a tree to maintain normal carbon metabolism has major impacts on its ability to survive stressful environmental conditions and bark beetle outbreaks (Huang et al. 2019; McDowell 2011; McDowell et al. 2008), and this study did not find that dinotefuran interfered with loblolly pine leaf-level photosynthetic processes. As such, dinotefuran application at a basal spray may be a valuable means of avoiding or mitigating southern pine beetle infestation in loblolly pine plantations.

This study did not detect statistically significant genotypic variation in P_{gmax} ($P = .20$), P_{net} ($P = .22$), or R_d ($P = .21$) (Table 2). In a previous study at this site, Blazier et al. (2018) observed that the 756 genotype had significantly greater light-saturated net photosynthesis than the 93 genotype at the leaf and canopy-levels. Our study also observed a tendency for the 756 genotype to exhibit greater average P_{net} and P_{gmax} than the 93 genotype, but this trend was not statistically significant (Table 2). This study also did not find a significant interaction of genotype \times insecticide for any of the light response variables ($P > .10$) (Table 1). Todorenko et al. (2020) observed genotypic variation in the

Table 2. Treatment and genotype least square means for loblolly pine maximum gross photosynthesis (P_{gmax} [$\mu\text{molm}^{-2} \text{s}^{-1}$]), light-saturated net photosynthesis (P_{net} [$\mu\text{molm}^{-2} \text{s}^{-1}$]), and dark respiration (R_d [$\mu\text{molm}^{-2} \text{s}^{-1}$]) with standard error in parentheses.

	P_{gmax}	P_{net}	R_d
Insecticide	9.15 (0.78)	7.08 (0.61)	0.56 (0.06)
Control	8.99 (0.78)	7.08 (0.61)	0.51 (0.06)
<u>Genotypes</u>			
LA	10.27 (0.95)	7.93 (0.74)	0.51 (0.08)
93	7.76 (0.95)	6.05 (0.74)	0.45 (0.08)
756	9.20 (0.95)	7.26 (0.74)	0.64 (0.08)

sensitivity of maize (*Zea mays* L.) genotypes to the systemic neonicotinoid insecticide thiamethoxam, but the results of this study suggest that dinotefuran does not interfere with the leaf-level carbon metabolism of loblolly pine, regardless of genotype.

Conclusions

Climate intensification is projected to increase tree physiological stress (McDowell et al. 2008; McNulty et al. 2019) and allow for bark beetle range expansion over the coming century (Duehl, Koch, and Hain 2011; Ungerer, Ayres, and Lobardero 1999; Williams and Liebhold 2002). Greater drought stress will likely increase tree vulnerability to bark beetle attacks (Dunn and Lorio 1993; Huang et al. 2019), which emphasizes the importance of insecticides to prevent or mitigate bark beetle outbreaks. Bark beetle infestation can be minimized by silvicultural management, insecticide application, and chemical pheromone release (Schowalter 2012), but some insecticides can be phytotoxic to *Pinus* species (Grosman and Upton 2006). Results of this study suggest that dinotefuran can be used to protect loblolly pine from bark beetles without risk of phytotoxic interference in leaf-level photosynthetic processes.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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