Hylastes spp. and their fungal associates in longleaf pine stands at Fort Benning GA

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Root feeding bark beetles of the genus *Hylastes* Erichson (Curculionidae, Scolytinae) are known to vector ophiostomatoid fungi including some important pathogens. In this study, pitfall traps baited with 95% ethanol and turpentine were placed in longleaf pine stands at Fort Benning, Georgia to attract *Hylastes* spp and other root-feeding beetles. Traps were visited weekly for 62 weeks, and ophiostomatoid fungi isolated by rolling on selective and non-selective media. *Hylastes tenuis* Eichhoff, *H. salebrosus* Eichhoff, and *H. porculus* Erichson were collected, in order of their relative abundance. Among the ophiostomatoid fungi isolated from these insects were *Grosmannia huntii* (Robinson-Jeffrey & Grinchenko) Zipfel, de Beer &Wingfield (syn *Leptographium huntii*), *L. procerum*(Kendrick) Wingfield, *L. terebrantis* Barras & Perry, and *L. serpens* (Goid.) Siem.. *Grosmannia huntii* and *L. serpens* have only recently been found in southeastern pines, but are known from Europe and other regions where the fungus is known to be introduced. *Hylastes* spp were found to be present throughout most of the year with population peaks in spring and fall, and to be vectoring ophiostomatoid fungi including potential exotic species.

Figure 1. Pitfall traps, L. Disassembled, R. In situ

Introduction

❖Root-feeding bark beetles (Curculionidae: Scolytinae), especially *Hylastes* spp. are frequently associated with ophiostomatoid fungi, including *Leptographium* spp. (Six, 2003)

❖ In the southeastern U.S., *Leptographium* spp. and increased beetle activity have been associated with loblolly pine decline (Eckhardt *et al.*, 2007) and longleaf pine decline (Otrosina *et al.*, 1999).

❖Previous studies in longleaf pine stands have investigated the pinaphagous beetle fauna (Hanula *et al.*, 2002; Sullivan *et al.*, 2003), but not the fungal associations with these vectors.

❖ In this study, *Hylastes* spp, and other pinophagous beetles were captured in pitfall traps and 'rolled' to isolate *Leptographium* spp and other ophiostomatoid fungi.

Figure 7. Perithecium of *G. huntii*.

Figure 4. The most frequently captured root-feeding beetles at Fort Benning, GA.

Color of bar beneath photo represents that species in subsequent Figures and Tables.

Figure 8. Conidiophores of *L. serpens*.

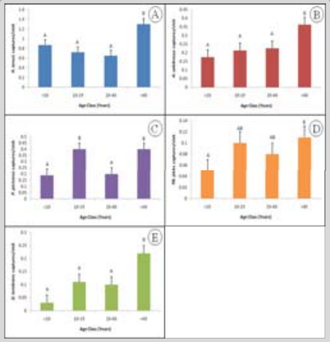

Figure 5. Mean number of captures per plot visit by age class, August 2006-August 2007. Columns within panes bearing the same letter are not significantly different at α=0.05. (A). *H. tenuis*, (B). *H. salebrosus*, (C). *P. picivorus*, (D). *Hb. pales*, (E). *D. terebrans*

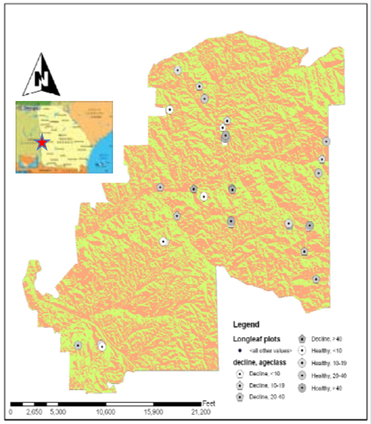

Figure 2. Plot locations at Fort Benning, GA

Methods and Materials

❖Pitfall traps (Figure 1) were installed at Fort Benning, GA (Figure 2) in stands of 4 age classes, <10 years, 10-19 years, 20-40 years, and >40 years.

❖Traps were baited with turpentine and 95% ethanol, with longleaf twig sections as additional bait and substrate, and were visited weekly, March 2006-May 2006 (10 weeks) and August 2006-August 2007 (52 weeks)

❖Captured insects were tallied, and then rolled on cycloheximide and streptomycin amended malt extract agar (CSMA, Hicks et al., 1980) and unamended 1% malt extract agar (MEA) (Figure 3).

❖Ophiostomatoid fungi recovered were identified by morphology

Figure 3. Cartoon of insect rolling

Results

❖The most frequently captured insects are depicted in Figure 4. For most species, capture numbers were highest in the oldest age class (Figure 5).

❖*Hylastes salebrosus* and *H. porculus*, similar in size and superficially similar in appearance, were pooled for most analyses. A post-hoc analysis of a subset of captured insects (n= 387) showed a shift in the ratio of these two species by season (Figure 6).

❖Seasonal peaks were different for all species (Figure 7) *Hylastes salebrosus* peaked in Spring, *H. tenuis* in Fall, *P. picivorus* in Summer, and *D. terebrans* (black turpentine beetle, BTB) in Fall/Winter.

❖Seven ophiostomatoid taxa were isolated from beetles (Table 1). Among the most abundant fungal species were *Grosmannia huntii*, *Leptographium procerum*, and *L. terebrantis* (Figures 7 and 8).

❖*Hylastes* spp. shared a similar mycota(χ^2_5= 5.58, p=0.35), as did regeneration weevils (χ^2_6= 8.14, p= 0.23). Black turpentine beetle did not share the same mycota with either of these groups (χ^2_{14}= 623.33, p< 0.0001).

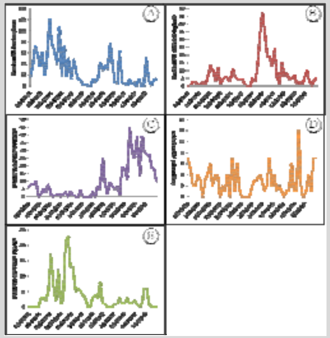

Figure 7. Weekly captures of root-feeding beetles in longleaf pine stands at Fort Benning, GA from 24 August 2006-26 August 2007.

(A). *H. tenuis*, (B). *H. salebrosus* and *H. porculus*, (C). *P. picivorus*, (D). *Hb. pales*, (E). *D. terebrans*

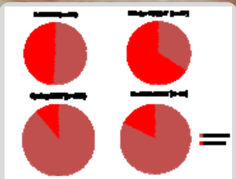

Figure 6. Comparison of *H. salebrosus* and *H. porculus* captures by season

Table 1. Incidence of fungi isolated from exoskeletons of root-feeding curculionids at Fort Benning, GA.

*Total includes 62 weeks of collection data,

Insect spp.(Total)	*L. procerum*		*L. terebrantis*		*L. serpens*		*G. aureum*		*G. huntii*		*Ophiostoma ips*-like		*Ophiostoma.* spp.		*Pesotum* spp.		Total by insect spp.	
	# Isolates	%	# Isolates	%	# Isolates	%	# Isolates	%	# Isolates	%	# Isolates	%	# Isolates	%	# Isolates	%	Total Isolates	% infested
Hylastes. tenuis (1726)	119	6.89%	95	5.50%	31	1.80%	2	0.12%	192	11.12%	19	1.10%	16	0.93%	129	7.47%	603	34.94%
H. salebrosus (596) *	11	1.85%	15	2.52%	1	0.17%	0	0.00%	26	4.36%	17	2.85%	3	1.01%	13	2.18%	86	14.43%
Pachylobius picivorus (567)	68	11.99%	47	8.29%	0	0.00%	0	0.00%	4	0.71%	3	0.53%	4	0.71%	1	0.18%	127	22.40%
Hylobius pales (247)	32	12.96%	31	12.55%	2	0.81%	0	0.00%	4	1.62%	3	1.21%	2	0.81%	3	1.21%	77	31.17%
Dendroctonus terebrans (215)	4	1.86%	5	2.33%	0	0.00%	26	12.09%	2	0.93%	33	15.35%	6	2.79%	10	4.65%	86	40.00%
Total by fungal spp.	234	23.90%	193	19.71%	34	3.47%	28	2.86%	228	23.29%	75	7.66%	31	3.17%	156	15.93%	979	29.22%

* Includes *H. salebrosus* and *H porculus*

Discussion

❖Vectors of ophiostomatoid fungi were present throughout the year, and different taxa peaked at different times of year. Few of the stands where traps were installed were exhibiting symptoms of decline when crowns were examined (Zanzot and Eckhardt, in preparation). However, root infections with *L. procerum* and *L. terebrantis* were common, and isolations from soil rare, suggesting that insects are the principle means of spreading the fungi in these stands. *Grosmannia huntii* was frequently isolated from *Hylastes* spp, but was recovered from roots of a single longleaf pine tree which subsequently died after expressing decline-like symptoms. This fungus has only recently been found in the southeastern U.S., and future research into pine decline in the southeast should include survey for these fungi. While the frequency of fungal recovery suggests a facultative relationship between insects and fungi, the distribution of fungal species suggests different niches for the insects most frequently captured.

Acknowledgments

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