

## Ecology of Root-feeding Beetles and Their Associated Fungi on Longleaf Pine in Georgia

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Environ. Entomol. 39(2): 415–423 (2010); DOI: 10.1603/EN09261

**ABSTRACT** Root-feeding beetles, particularly of the curculionid subfamilies Scolytinae and Molytinae, are known to be effective vectors of Ophiostomatoid fungi. Infestation by these insects and subsequent infection by the Ophiostomatoid fungi may play an important role in accelerating symptom progression in pine declines. To examine the relationship between beetles and fungi in longleaf pine stands, root-feeding curculionids were collected in pitfall traps baited with ethanol and turpentine for 62 wk, and Ophiostomatoid fungi were isolated from their body surfaces. The most abundant root-feeding beetles captured were *Hylastes tenuis*, *H. salebrosus*, *Pachylobius picivorus*, *Hylobius pales*, and *Dendroctonus terebrans*. The number of insects captured peaked in spring and fall, although peaks for different insect taxa did not coincide. The most frequently isolated fungi were *Grosmannia huntii*, *Leptographium procerum*, *L. terebrantis*, and *L. serpens*. Other Ophiostomatoid fungi recovered included *Ophiostoma* spp. and *Pesotum* spp. Insect infestation data suggest that *Hylastes* spp. share an ecological niche, as do *Hb. pales* and *P. picivorus*, because the ratios of their fungal symbionts were similar. The fungi associated with *D. terebrans* suggest that it did not share habitat with the other principle vectors.

**KEY WORDS** bark beetles, regeneration weevils, Ophiostomatoid fungi, *Pinus palustris*, *Leptographium* spp

Bark beetles and regeneration weevils (Coleoptera: Curculionidae, subfamilies Scolytinae and Molytinae, respectively) live in a close association with Ophiostomatoid fungi (Ascomycota: Ophiostomatales) (Wingfield et al. 1993). These fungi are commonly referred to as blue stain fungi because of the cosmetic damage some species cause in logs and lumber (Seifert 1993). Most members bear their meiotically or mitotically derived spores in slimy matrices suitable for transfer by insects (Malloch and Blackwell 1993), and they include the sexual genera *Ophiostoma* Sydow and P. Sydow, *Ceratocystiopsis* Upadhyay and Kendrick, and *Grosmannia* Goidanich, as well as the asexual form-genera *Pesotum* Crane and Schoknecht emend Okada and Seifert, *Sporothrix* Hektoen and Perkins, *Hyalorhinochlaediella* Upadhyay and Kendrick, and *Leptographium* Lagerberg and Melin (Zipfel et al. 2006).

Some members of the genus *Grosmannia* (anamorph: *Leptographium*, sensu Zipfel et al. 2006) are known for their role in forest diseases and declines. Black stain root disease affects several conifer species in the Pacific Northwest and is caused by *G. wagneri* (Kendrick) Zipfel, de Beer and Wingfield (Wagner and Mielke 1961, Cobb 1988). The causal fungus is vectored by bark beetles including *Hylastes nigrinus*

(Mannerheim) (Witcosky et al. 1986) and *H. macer* LeConte (Goheen and Cobb 1978), and it is the only *Leptographium* sp. known to be a primary pathogen of conifers. In South Africa, *L. serpens* (Goidanich) Siemaszko, *L. lundbergii* Lagerberg and Melin and other Ophiostomatoid fungi have been isolated from the roots of dying *Pinus* spp. after early infestation by the European bark beetles *H. angustatus* (Herbst) and *Hylurgus ligniperda* (Fabricius) (Wingfield et al. 1988; Zhou et al. 2001, 2002). *Pinus* spp. have been introduced in the southern hemisphere, in contrast with northern hemisphere plantations where native pine species are typically planted. Likewise, exotic pinophagous bark beetles and their associated fungi have been introduced into other southern hemisphere countries; notably, *H. ater* (Paykull) has been found in New Zealand accompanied by *G. huntii* (Robinson-Jeffrey and Grinchenko) Zipfel, de Beer and Wingfield, *L. procerum* (Jacobs and Wingfield 2001), and *O. ips* (Reay et al. 2002). In Chile, *H. ater* and *Hg. ligniperda* have been found carrying *G. huntii*, *O. ips*, and other Ophiostomatoid fungi (Zhou et al. 2004).

In the northeastern United States, white pine (*Pinus strobus* L.) root decline has been linked to the presence of *L. procerum* (Kendrick) Wingfield, *L. terebrantis* Barras and Perry (Wingfield 1986, Alexander et al. 1988) and their associated curculionid vectors including the weevils *Hylobius pales* (Herbst) and *Pis-*

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*sodes nemorensis* Germar, and a suite of scolytine beetles (Nevill and Alexander 1992). The same fungi have been implicated in red pine (*P. resinosa* Aiton) decline (Klepzig et al. 1991). The associated vectors were more typically bark beetles, *D. valens* LeConte and *H. porculus* Erichson (Klepzig et al. 1995), although the weevils *Pachylobius picivorus* (Germar), *Hb. pales*, and *Hb. radialis* Buchanan were also found to be infested with *L. procerum*, *L. terebrantis*, and other Ophiostomatoid fungi including *O. ips* and *G. huntii* (Klepzig et al. 1991).

In the southeastern United States, Ophiostomatoid fungi and their vectors have been found to be important contributing factors in loblolly pine (*P. taeda* L.) decline (Eckhardt et al. 2007). In loblolly pine stands experiencing decline, *Hb. pales*, *P. picivorus*, and *Ps. nemorensis* were captured, as well as southern scolytines of the same guild: *H. salebrosus* Eichhoff, *H. tenuis* Eichhoff, and *D. terebrans* (Olivier). These insects were found to be infested with *L. procerum* and *L. terebrantis*, as well as *L. serpens* (Eckhardt et al. 2007). In pathogenicity tests, *L. serpens* and *L. terebrantis* had greater effects on seedling growth than *L. procerum*, with *L. serpens* producing larger lesions than *L. procerum* or *L. terebrantis* in seedlings and mature trees (Eckhardt et al. 2004b). Infestation with *Lep-tographium* spp. was shown to increase brood size in *Hylastes* spp., which in turn was effective in inoculating the fungi into uninfected root sections (Eckhardt et al. 2004a).

Longleaf pine (*P. palustris* Miller) has been reported to experience decline-like symptoms in the southeastern United States (Otrosina et al. 1999). Although a pyrophytic species (Frost 2006), mature longleaf pine were dying after prescription burning. A survey of the roots showed *L. procerum* and *L. terebrantis*, but no connection was made to insect vectors at that time (Otrosina et al. 1999). The effects of fire on insect populations in longleaf pine stands have been examined after wildfire (Hanula et al. 2002) and prescription fire (Sullivan et al. 2003). *Hylastes* spp. and other phloem-feeders were more abundant in unburned areas than in burned sites after a wildfire in Florida, whereas the trend with xylem-feeding scolytines was an increase in populations with burn severity (Hanula et al. 2002). In a similar study, applying prescription burns of differing intensity in southern Alabama, the opposite trend was observed in phloephagous scolytines (Sullivan et al. 2003). Some of the regeneration weevils were examined for the presence of Ophiostomatoid fungi in the Florida study and showed the presence of *L. procerum* and *L. terebrantis* (Hanula et al. 2002).

Relatively little is known regarding the occurrence or biology of the insects that infest pine roots or the fungi that they may carry. For this reason, and associated with a concern regarding the decline, the distribution and abundance of root-feeding bark beetles and regeneration weevils, and their phoretic Ophiostomatoid fungi, were studied at Fort Benning Military Reservation, GA. The study site is located in the sandhills ecoregion near the Fall Line separating the

coastal plain from the piedmont region in west central Georgia. Fort Benning (FB) maintains an active prescription burning program to enhance longleaf pine habitat. In addition to investigating seasonal differences in beetle populations, the patterns of association of the fungi that they carry were considered.

## Materials and Methods

**Plot Setup and Collection of Pinophagous Beetles.** Thirty-two 0.07-ha plots were installed at FB, in Muscogee and Chattahoochee Counties in Georgia, and Russell County, AL. Plot design followed the protocol of the USDA Forest Service's Forest Inventory and Analysis program (Dunn 1999). Each total plot was an array of a central plot and three subplots: all circles of 7.62 m. Plots were blocked into four age classes (<10, 10–19, 20–40, and >40 yr at the beginning of the study, February 2006), with eight replicates of each age class. Plot locations were selected by querying for appropriate stand type and age class in ArcGIS 3.2a (ESRI 1996), and ~15 random points were generated per age class. Final plot locations were selected by ground truthing of the random points for appropriate stand conditions (longleaf pine dominant, accurate age classification). Although plots were occasionally situated within a single stand, no two plots were closer than 150 m.

Pitfall traps were placed in each outlying subplot (3/plot) to collect vector beetles. The traps (adapted from Klepzig et al. 1991) were constructed of sections of PVC tubing 10 cm diameter and 20 cm long with a fixed cap at the bottom end and a loose cap at the top. Eight radial holes (6 mm diameter) were drilled to allow insects to enter and fall into a cup placed inside the trap. A plastic skirt was fitted on the trap to reduce the risk of flooding (Menard 2007), such that the entry holes on the trap were within 5 cm of ground level. Steam-distilled southern pine turpentine (Hercules, Wilmington, DE) and 95% ethanol (in two 8-ml vials) were used as baits. Three sections of longleaf pine twig, ≈5 cm long by 2 cm diameter, were also placed in the cup as substrate for the captured insects and as additional bait.

Pitfall trap collection was performed weekly from 4 March 2006 to 5 May 2006 (10 collections) and from 24 August 2006 to 26 August 2007 (52 collections). Thus, data were collected for two spring seasons (2006 and 2007) and one continuous year (2006–2007). During each trap collection, the contents of the pitfall traps were transferred to a clean specimen cup and stored at 4°C for laboratory analysis. The trap cups were sprayed with 70% ethanol to disinfect them and swabbed with liquid Teflon (Northern Products, Woonsocket, RI) to deter insects from crawling out of the traps. Fresh twig sections were replaced in the trap cups. The turpentine and ethanol baits were evacuated and refilled. In the laboratory, insects were sorted from each specimen cup and identified to species and tallied.

Insect numbers were pooled at the plot and species levels throughout the course of the sampling period

**Table 1.** Most frequently captured root-feeding beetles, summed by stand age class at Fort Benning, GA

Insect species	Age class (yr)				Total
	<10	10–19	20–40	>40	
<i>H. tenuis</i>	409	332	369	616	1,726
<i>H. salebrosus</i> <sup>a</sup>	93	113	168	222	596
<i>P. picivorus</i>	82	184	122	179	567
<i>Hb. pales</i>	58	67	54	68	247
<i>D. terebrans</i>	16	55	47	97	215

<sup>a</sup> Includes *H. porculus*.

and effects of tree age class and sampling week tested using repeated-measures analysis with the first-order autoregressive model (AR1) for the covariance structure (PROC MIXED; SAS Institute 2003).

**Effects of Weather Variables.** Effects of temperature and precipitation were assessed using weather data from the National Climatic Data Center (<http://cdo.ncdc.noaa.gov/dly/DLY>). Data from the Columbus Airport weather station were used, because data from the FB weather station during the study period are incomplete. Average weekly maxima and minima were calculated from daily maxima and minima. Weekly precipitation was summed from the daily precipitation data during the period between collection dates. Weekly totals of *H. tenuis*, *H. salebrosus*, *Hb. pales*, *P. picivorus*, and *D. terebrans* were pooled by plot for correlation analysis with temperature and precipitation (PROC CORR; SAS Institute 2003). To assess seasonal effects, weekly capture numbers for each plot and week were compared by coding 13-wk periods with dummy variables and analysis (PROC CORR and PROC GLM; SAS Institute 2003). For analysis of variance (ANOVA), means were separated using the Tukey-Kramer procedure.

**Infestation With Ophiostomatoid Fungi.** Each captured beetle of interest was rolled across the surface of one petri plate of malt extract agar amended with cycloheximide and streptomycin (CSMA) (Hicks et al. 1980) and one plate of unamended 2% malt extract agar (wt:vol, MEA) to isolate fungal propagules. Cycloheximide tolerance is a character shared by most Ophiostomatoid fungi; unamended MEA was included to allow for growth of rare cycloheximide-intolerant species. Plates were inspected after 5 d and periodically thereafter for 4 wk for the presence of Ophiostomatoid fungi. These fungi were serially transferred

via spore droplets or hyphal tips to CSMA until pure cultures had been obtained. The fungi were transferred to MEA for storage before species determination. Identifications were made based on microscopic examination of morphological and cultural characters as described in Jacobs and Wingfield (2001) for *Lep-tographium* spp. Other Ophiostomatoid fungi (*Pesotum* spp., *Ophiostoma* spp.) were identified to genus and pooled for subsequent study.

The distribution of fungi on insects was compared using  $\chi^2$  tests (PROC FREQ; SAS Institute 2003). The correlation between fungal isolation from beetles and weather variables was tested in a similar manner.

## Results

**Incidence of Pinophagous Beetle Activity and Seasonal Effects.** A total of 3,351 root-feeding beetles were captured (Table 1). Four species of scolytine bark beetles (*H. tenuis*, *H. salebrosus*, *H. porculus*, and *D. terebrans*) and two species of molytine weevils (*Hb. pales* and *P. picivorus*) were captured most frequently. Voucher specimens of these beetles have been deposited in the Louisiana State Arthropod Museum, Baton Rouge, LA, as part of the dissertation work of the primary author. Other scolytines captured included *Ips avulsus* (Eichhoff) ( $n = 67$ ), *I. grandicollis* (Eichhoff) ( $n = 34$ ), *Xyleborinus saxesenii* (Ratzeburg) ( $n = 162$ ), and *Xylosandrus crassiusculus* (Motschulsky) ( $n = 84$ ).

*Hylastes tenuis* was the most frequently captured insect, with 1,726 individuals collected. A post hoc examination of 250 randomly selected *Hylastes* individuals did not show any specimens of *H. opaculus* Erichson, a morphologically similar but exotic insect. Except where indicated below, data for *H. salebrosus* and *H. porculus* were pooled, because both were tallied as *H. salebrosus* during the collection period. A post hoc examination of intact captured specimens showed 25% (97/385) of beetles originally identified as *H. salebrosus* were *H. porculus*. The ratio of these species varied by season.

Week of capture was a significant factor in all species tested by repeated-measures analysis (Table 2). Stands in the >40-yr age class yielded more insects than the <10-yr age class in all species tested, and were higher than all other ages for *H. tenuis*, *H. salebrosus*, and *D. terebrans* (Fig. 1).

**Table 2.** Summary statistics for repeated-measures analysis for effects of age class and week of capture at Fort Benning, GA, Aug. 2006 to Aug. 2007

Species	Overall model			Age class		Week	
	NMLRT <sup>a</sup>	P	AIC <sup>b</sup>	F (df)	P	F (df)	P
<i>H. tenuis</i>	130.84	<0.0001	6148	7.39 (3,28)	0.001	8.60 (50,1550)	<0.0001
<i>H. salebrosus</i> <sup>c</sup>	53.2	<0.0001	3598	3.67 (3,28)	0.02	4.87 (50,1550)	<0.0001
<i>P. picivorus</i>	81.47	<0.0001	4056	5.11 (3,28)	0.006	4.19 (50,1550)	<0.0001
<i>Hb. pales</i>	1.85	0.17	1349	1.97 (3,28)	0.14	1.74 (50,1550)	0.001
<i>D. terebrans</i>	77.52	<0.0001	2635	5.10 (3,28)	0.006	2.43 (50,1550)	<0.0001

<sup>a</sup> Null model likelihood ratio test for overall model,  $\chi^2_1$  distributed.

<sup>b</sup> Akaike information criterion.

<sup>c</sup> Includes *H. porculus*.

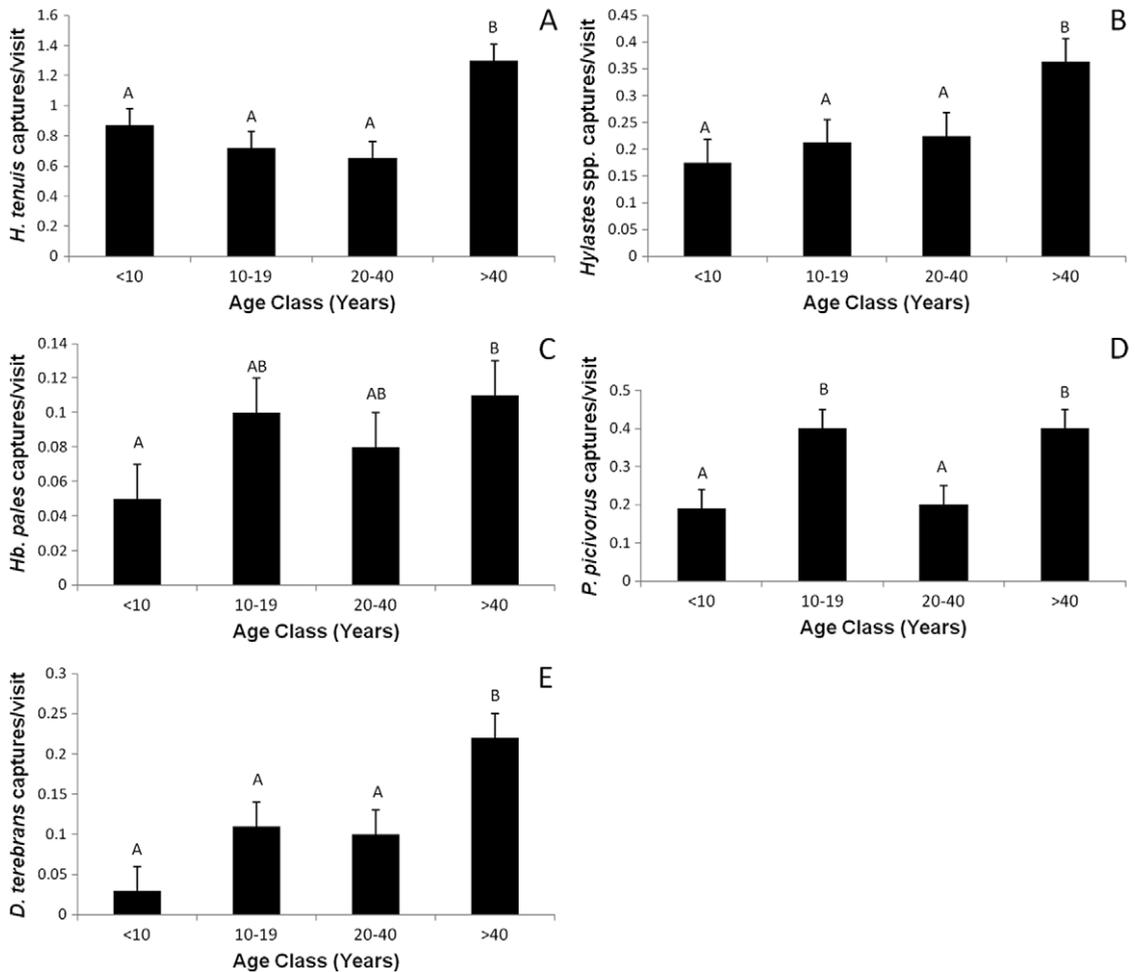


Fig. 1. Mean number of captures per plot visit by age class at Fort Benning, GA, August 2006 to August 2007. Columns within panes bearing the same letter are not significantly different at  $P = 0.05$ . Mean separation by Tukey Kramer method. (A) *H. tenuis*, (B) larger *Hylastes* spp. (*H. salebrosus* and *H. porculus*), (C) *P. picivorus*, (D) *Hb. pales*, and (E) *D. terebrans*.

Weekly temperature maxima ranged from 9 to 37°C, with an average of 25°C, and the weekly temperature minima ranged from -4.4 to 25°C, with an average of 13°C during the sampling period. Weekly precipitation peaked at 13.2 cm and averaged 2.3 cm. Insects were captured in all weeks except during 3 wk (February 2007) when temperature minima were below freezing. Weekly precipitation did not correlate with numbers of insects captured, but annual precipitation was below average overall.

Numbers of captured beetles peaked in spring and fall, with a maximum of 100 beetles captured in spring 2006 and 149 in fall 2006. All species were present during the spring trapping season in both years (Figs. 2 and 3). The number of insects captured, dropped to zero for 3 wk in February 2007, corresponding to a period of low temperature. *D. terebrans* was negatively correlated with weekly temperature maxima and minima ( $\rho = -0.368$ ,  $P = 0.003$  and  $\rho = -0.373$ ,  $P = 0.003$ , respectively), with a population peak during winter trapping ( $\rho = 0.438$ ,  $P = 0.0004$ ). Although *Hylastes*

spp. peaked in spring and fall, the numbers of *H. tenuis* were higher in the fall ( $\rho = 0.552$ ,  $P < 0.0001$ ), and the number of *H. salebrosus* peaked in the spring ( $\rho = 0.633$ ,  $P < 0.0001$ ; Fig. 2A and B). The numbers of *P. picivorus* captured were positively correlated with temperature maxima and minima ( $\rho = 0.667$ ,  $P < 0.0001$  and  $\rho = 0.643$ ,  $P < 0.0001$ , respectively) and were higher during the summer ( $\rho = 0.745$ ,  $P < 0.0001$ ; Fig. 2C) and lower in fall and winter ( $\rho = -0.285$ ,  $P = 0.0249$  and  $\rho = -0.420$ ,  $P = 0.0007$ , respectively). The number of *Hb. pales* captured was numerically higher, but not significantly so, in spring ( $\rho = 0.214$ ,  $P = 0.099$ ).

Comparisons between numbers of insects captured in spring 2006 and 2007 (Fig. 3) were similar for *D. terebrans* ( $F = 7.37$ ;  $df = 1,8$ ;  $P = 0.03$ ), and *H. salebrosus* (including *H. porculus*,  $F = 5.53$ ;  $df = 1,8$ ;  $P = 0.04$ ). Comparisons of insects captured in the two spring seasons were dissimilar for *H. tenuis* ( $F = 2.17$ ;  $df = 1,8$ ;  $P = 0.14$ ), *Hb. pales* ( $F = 0.03$ ;  $df = 1,8$ ;  $P = 0.85$ ), and *P. picivorus* ( $F = 0.01$ ;  $df = 1,8$ ;  $P = 0.92$ ).

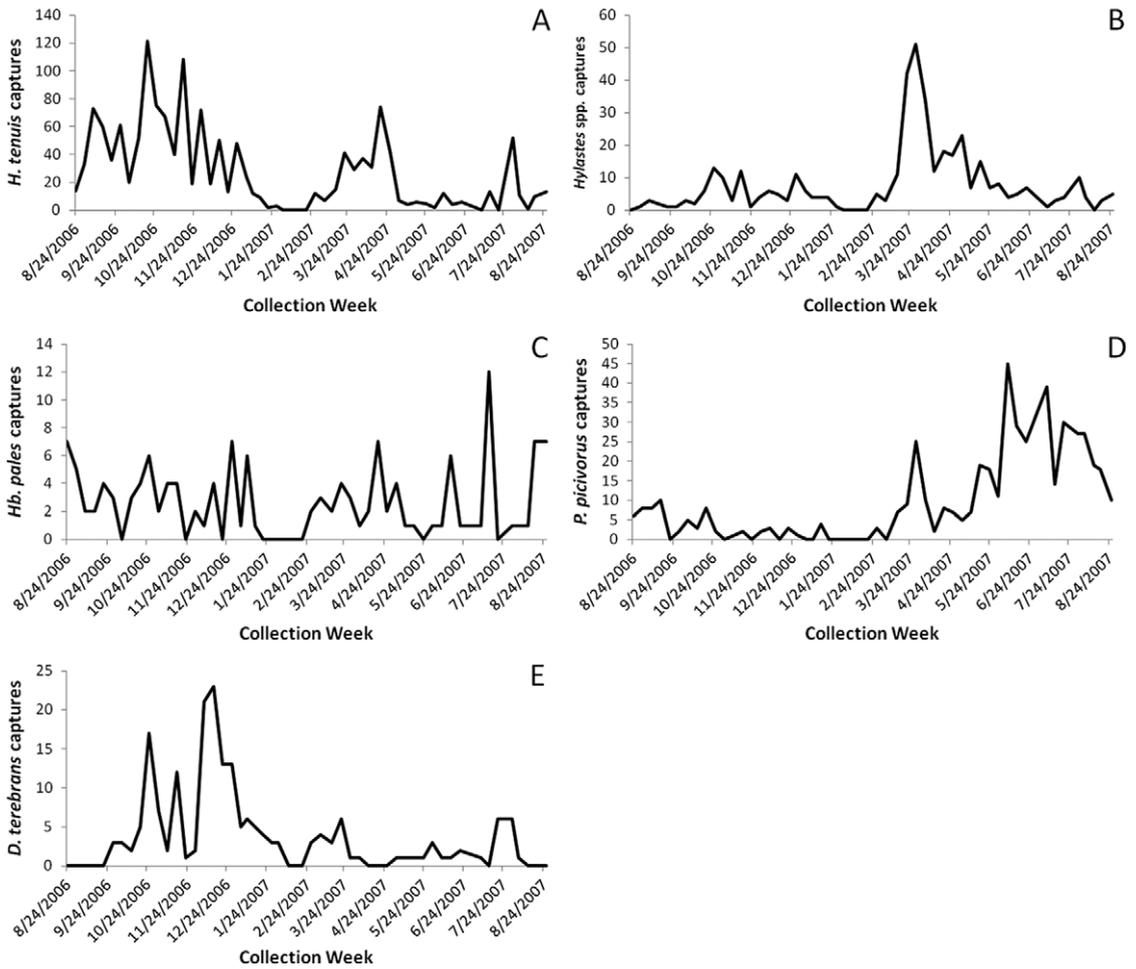


Fig. 2. Weekly captures of root-feeding beetles in longleaf pine stands at Fort Benning, GA, from 24 August 2006 to 26 August 2007. (A) *H. tenuis*, (B) larger *Hylastes* spp. (*H. salebrosus* and *H. porculus*), (C) *P. picivorus*, (D) *Hb. pales*, and (E) *D. terebrans*.

The subset of *H. salebrosus* that were examined in the post hoc diagnoses showed the ratio of *H. salebrosus*:*H. porculus* to vary throughout the years. Spring and summer ratios ranged from 90% *H. salebrosus* (166:20 Hs:Hp) to 73% (60:13 Hs: Hp), but in winter, the ratio shifted to 65% *H. porculus* (16:31 Hs:Hp). Differences between seasonal ratios were significant ( $\chi^2 = 80.1$ ;  $df = 4$ ;  $P < 0.0001$ ).

**Infestation With Ophiostomatoid Fungi.** The predominant Ophiostomatoid fungi recovered from the beetle species considered in this study were *L. procerum*, *L. terebrantis*, *G. huntii*, and *Pesotum* spp. (Table 3). Overall, 27% of the major scolytine and molytine insects yielded isolates of Ophiostomatoid fungi, suggesting that these fungi are facultatively associated with their insect vectors. *L. procerum* (24%), *G. huntii* (23%), *L. terebrantis* (20%), and pooled *Pesotum* spp. (16%) were the most frequently observed fungi-infesting insects (Table 3). The distribution of fungi was similar between the pine regeneration weevils, *H. pales*, and *P. picivorus* ( $\chi^2 = 8.14$ ;  $df = 6$ ;  $P = 0.23$ ), and

between *Hylastes* spp. ( $\chi^2 = 5.58$ ;  $df = 5$ ;  $P = 0.35$ ). The mycota of *D. terebrans* differed from the other most commonly captured beetles ( $\chi^2 = 623.33$ ;  $df = 14$ ;  $P < 0.0001$ ). *D. terebrans* rarely yielded isolates of *L. terebrantis* and had a higher frequency of isolates resembling *G. aureum* and *O. ips* but probably undescribed fungal species (M. J. Wingfield, personal communication).

*Leptographium procerum* isolates from insects were positively correlated with high weekly temperature minima ( $\rho = 0.197$ ,  $P = 0.0297$ ; Table 4) and were most frequently collected during fall 2006 ( $\rho = 0.307$ ,  $P = 0.0151$ ). Fewer *L. procerum* isolates were collected during winter ( $\rho = -0.279$ ,  $P = 0.0278$ ), and *L. terebrantis* isolates increased in spring ( $\rho = 0.375$ ,  $P = 0.0027$ ) and decreased in summer and winter ( $\rho = -0.403$ ,  $P = 0.0012$  and  $\rho = -0.258$ ,  $P = 0.043$ , respectively). *Ophiostoma* spp. isolates (excluding *O. ips*-like) were collected more frequently in spring ( $\rho = 0.337$ ,  $P = 0.0075$ ) and less frequently in winter ( $\rho = -0.301$ ,  $P = 0.013$ ). *Ophiostoma ips*-like isolates were less abundant during periods

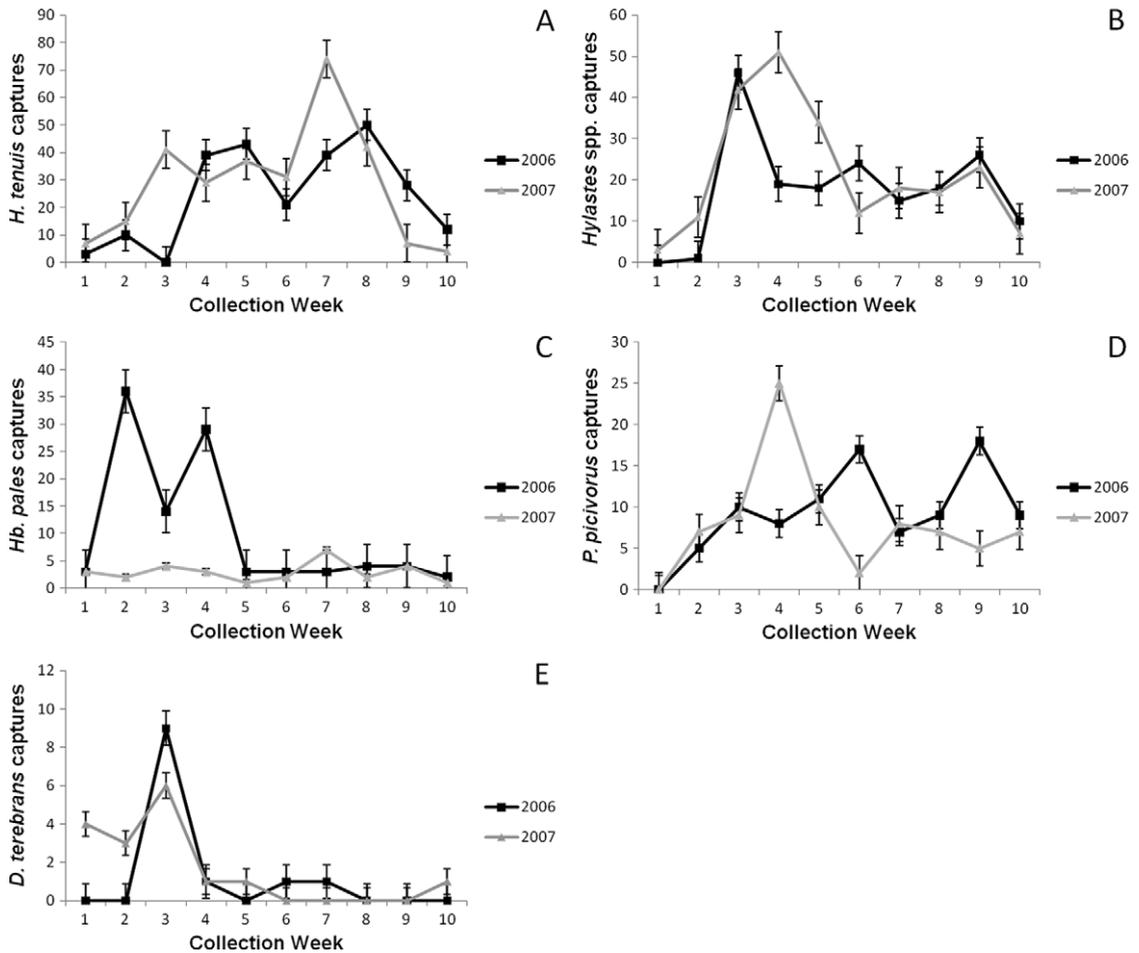


Fig. 3. Weekly captures ( $\pm$ SEM) of root-feeding beetles in longleaf pine stands at Fort Benning, GA, spring (early March to early May) 2006 and 2007. (A) *H. tenuis*, (B) larger *Hylastes* spp. (*H. salebrosus* and *H. porculus*), (C) *P. picivorus*, (D) *Hb. pales*, and (E) *D. terebrans*.

of temperature minima ( $\rho = -0.323$ ,  $P = 0.0104$ ). The *G. aureum*-like fungus was more frequently isolated in fall ( $\rho = 0.259$ ,  $P = 0.0417$ ).

### Discussion

The results presented here showed that root-feeding beetles are active throughout most of the year at

FB and that they carry a suite of Ophiostomatoid fungi, including species previously unreported in the region. Several studies have examined the bark beetle (excluding southern pine beetle) and regeneration weevil fauna of southeastern forests (Fatzinger, 1985; Sullivan et al. 2003; Campbell et al. 2008a, b). However, relatively few studies (Barnard et al. 1991, Otrrosina et al. 1999) have considered the Ophiostomatoid fungi

Table 3. Incidence of fungi isolated from exoskeletons of root-feeding curculionids at Fort Benning, GA

Insect spp. (spring 2006, spring 2007, total) <sup>a</sup>	<i>Lp</i> <sup>b</sup>	<i>Lt</i>	<i>Ls</i>	<i>Ga</i>	<i>Gh</i>	<i>Oi</i>	<i>O. spp.</i>	<i>P. spp.</i>	Total by insect spp.	Percent infestation
<i>D. terebrans</i> (11,15,215)	4	5	0	26	2	33	6	10	86	40
<i>H. tenuis</i> (242,286,1726)	119	95	31	2	192	19	16	129	603	35
<i>H. salebrosus</i> (166,205,596) <sup>c</sup>	11	15	1	0	26	17	3	13	86	14
<i>P. picivorus</i> (91,76,567)	68	47	0	0	4	3	4	1	127	22
<i>Hb. pales</i> (98,26,247)	32	31	2	0	4	3	2	3	77	31
Total by fungal spp.	234	193	34	28	228	75	31	156	979	

<sup>a</sup> Total includes both springs (early Mar. to early May), and fall 2006 to fall 2007 (late Aug. 2006 to late Aug. 2007) data.

<sup>b</sup> *Lp*, *L. procerum*; *Lt*, *L. terebrantis*; *Ga*, *G. aureum*-like; *Ls*, *L. serpens*; *Gh*, *G. huntii*; *Oi*, *O. ips*-like; *O. spp.*, other pooled *Ophiostoma* spp.; *P. spp.*, pooled *Pesotum* spp.

<sup>c</sup> Includes *H. porculus*.

**Table 4.** Correlation table between weather variables and root-feeding-beetle-derived fungal isolates at Fort Benning, GA

	<i>Lp</i> <sup>a</sup>	<i>Lt</i>	<i>Ls</i>	<i>Gh</i>	<i>Ga</i>	<i>Oi</i>	<i>O. spp.</i>	<i>P. spp.</i>	Insects
Weekly max temp	0.19736	-0.00071	-0.04624	0.00605	-0.06092	-0.23445	0.11978	-0.09182	0.10169
	0.1242	0.9956	0.7212	0.9628	0.6381	0.0666	0.3538	0.4779	0.4316
Weekly min temp	0.27638*	-0.02521	-0.01137	0.03184	-0.03488	-0.32334*	0.06045	-0.06965	0.07036
	0.0297	0.8458	0.9301	0.8059	0.7878	0.0104	0.6407	0.5906	0.5869
Weekly precipitation	0.12075	-0.12445	-0.00524	-0.12050	0.02175	-0.13169	-0.13810	-0.15595	0.16807
	0.3498	0.3352	0.9678	0.3509	0.8668	0.3076	0.2844	0.2261	0.1916

For each cell, the upper value is the Pearson correlation coefficient ( $\rho$ ) and the lower value is the probability,  $P$ , of the absolute value of  $\rho = 0$ . Significant values ( $P < 0.05$ ) of  $\rho$  are indicated by asterisks.

<sup>a</sup> *Lp*, *L. procerum*; *Lt*, *L. terebrantis*; *Ga*, *G. aureum*-like; *Ls*, *L. serpens*; *Gh*, *G. huntii*; *Oi*, *O. ips*-like; *O. spp.*, other pooled *Ophiostoma* spp.; *P. spp.*, pooled *Pesotum* spp.

associated with these insects. In addition, very few studies (Hanula et al. 2002, Eckhardt et al. 2007, Menard 2007) have directly considered the relationship between phloeophagous root-feeding curculionids and their mycota in the southeastern United States.

Beetles were present during 59 of 62 wk of sampling, indicating that root-feeding beetles and their fungi are active at FB almost year-round. In previous studies of the fungi associated with root-feeding beetles, collections were restricted to the spring, from early March to May (Eckhardt et al. 2007, Menard 2007). The results presented here indicate that year round sampling is necessary to assess population peaks in the curculionid fauna.

Numbers of insect captured in this study were lower than those in studies using other trap designs such as stovepipe (Fatzinger 1985) or multiple funnel traps (Hanula et al. 2002, Sullivan et al. 2003). However, this study required living insects to maximize fungal isolation success, and this precluded the use of lethal traps. Therefore, direct comparisons should be made with those in loblolly pine with similar plot layouts, identical traps, and performed in the same area (Eckhardt et al. 2007, Menard 2007).

In general, the numbers of vector captures were similar to predicted asymptomatic plots in studies of loblolly pine decline (Eckhardt et al. 2007, Menard 2007). The number of *H. tenuis* captured was greater than the larger *Hylastes* spp., in contrast with other studies where the larger insects were predominant (Bauman 2003, Eckhardt et al. 2007) or similar in number (Menard 2007). *P. picivorus* outnumbered *Hb. pales* by  $\approx 5:2$ . The natural range of these weevils largely overlap, with *Hb. pales* being predominant throughout much of that area, but the ratios are known to shift in favor of *P. picivorus* in the southeast (Nord et al. 1984). Fluctuations in the ratio of these weevils have been found in other studies in the southeastern United States, with *P. picivorus* more abundant in some cases (Sullivan et al. 2003, Menard 2007), *Hb. pales* in others (Hanula et al. 2002), or fluctuating between the two species (Eckhardt et al. 2007). Although strong correlations with weather were observed for *D. terebrans* and *P. picivorus*, the single year's data are not definitive, as has been shown in other year-round studies (Sullivan et al. 2003). However, the occurrence of a winter peak in *D. terebrans* and *H. porculus* in the southeast re-emphasizes the need for year-

round surveys for these species. Also, although little is known of the biologies of *H. porculus* and *H. salebrosus*, data presented here suggest that their population peaks are not synchronous in western Georgia.

The overall occurrence of Ophiostomatoid fungi in this study was lower than in previous studies (Eckhardt et al. 2007). The proportion of fungal isolations from insects suggests niche overlap between the regeneration weevil species and between *Hylastes* spp. The rarity of *L. terebrantis* on its eponymous host was unexpected, as a previous study has shown 90% infestation of this fungus on *D. terebrans* (Eckhardt et al. 2007) compared with  $\approx 2\%$  in this study. *G. huntii* and *L. serpens* were isolated more frequently from *Hylastes* spp. *L. serpens*, previously reported from loblolly pine and associated insects in Alabama (Eckhardt et al. 2007), was isolated infrequently from insects (<1% of all major vectors and 3.5% of all insect isolates).

*Pesotum* spp. (like *Ophiostoma* and *Leptographium* spp.) are cycloheximide tolerant fungi formerly assigned to the form-genus *Graphium*. Their biology is similar to *Ophiostoma* and *Leptographium* (Okada et al. 1998, Harrington 2005). Few *Pesotum* spp. are known to be pathogenic, although members of the *Ophiostoma ulmi* complex produce *Pesotum* anamorphs and represent an important exception (Upadhyay 1981). Their role has been ignored in previous studies with this pathosystems (Otrošina et al. 1999, Eckhardt et al. 2007), and a rigorous attempt to identify isolates to species level was not pursued in this study. Future studies may reinforce the observed niche differences between insect taxa in these fungi as well, and they might play an important role in longleaf pine decline.

*Grosmannia huntii* was the fungus most frequently found on *Hylastes* spp., and this is the first time that it has been recorded from the southeastern United States. This fungus is morphologically similar to *L. serpens*, and the two fungi might have been confused in the past (Eckhardt et al. 2007.). Both fungi have only recently been found in the southeastern United States, and the dominance of these fungi on root feeding insects suggests that they may play some role in the ecology of their vectors. Furthermore, the fact that *L. serpens* has been implicated in tree diseases in the past (Wingfield et al. 1988, Eckhardt et al. 2004a) suggests that they deserve further study.

Some of the fungi isolated from insects in this study most likely represent novel taxa that bear only a resemblance to known species. These include isolates that have been treated as *O. ips* and *G. aureum*. There is also some indication (unpublished data) that isolates of *G. huntii* recovered in this study are different to this fungus typified by isolates of the fungus originally described from lodgepole pine (*P. contorta* Douglas) in Canada (Robinson-Jeffrey and Grinchenko 1964). Although fungal identifications presented in this study are sufficient to understand patterns of association with root feeding insects, more accurate identifications in the future will refine our understanding of the relationships of the Ophiostomatoid fungi with insects for which the ecology has been minimally studied in the past.

Fungal recovery from insects varied by season, and in some cases, these trends followed host affinities. For example, the *O. ips*-like isolates were most commonly isolated from insects captured in winter, which is consistent with the fact that this fungus is closely associated with *D. terebrans* that was also captured most frequently in winter months. In other cases, fungal isolations seemed to be less dependent on the insect vectors. For example, *L. terebrantis* isolations were most common in spring, although the insects that carry this fungus were most commonly captured in fall. Whether temperature effects on seasonal changes are caused by temperature directly or may result from seasonal changes in host phenology or vector behavior cannot be tested from the results of this study. However, the results suggest that a further examination of seasonal effects on the occurrence of Ophiostomatoid fungi with their insect vectors deserves further study.

#### Acknowledgments

We thank the Department of Defense for funding this research and the staff of the Land Management Branch, Fort Benning Military Reservation, in particular J. Parker, for support. M. Wingfield (University of Pretoria, Pretoria, South Africa) and S. Enebak (Auburn University, Auburn, AL) reviewed the manuscript and provided invaluable comments. Prior studies by R. Menard provided a foundation for this study. We further acknowledge the technical support of several undergraduate research assistants and T. Garren-Grubbs for archiving insect-derived fungal isolates.

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Received 16 September 2009; accepted 18 January 2010.