NOTE

Inability to Rear Bark Beetles (Coleoptera: Scolytidae) on Tissue-Cultured Host Pine Callus[1]

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Rearing conifer-infesting bark beetles (Coleoptera: Scolytidae) outside of their host trees has been attempted with varying degrees of success using several types of semi-artificial media. An effective diet would provide a tool for examining the influence of tree secondary metabolites and beetle-associated fungi on the within-tree developing brood. The three southern *Ips* spp. *(I. avulsus* (Eichhoff), *I. calligraphus* (Germar), and *I. grandicollis* (Eichhoff)) and *Dendroctonus frontalis* Zimmermann have all been successfully reared on yeast-fortified ground phloem diets (Yearian, W. C. and R. C. Wilkinson. 1967. Fla. Entomol. 50: 43-45, Bridges, J. R. 1979. J. Ga. Entomol. Soc. 14: 278-279) and *D. frontalis* has been reared on tissue-cultured loblolly pine, *Pinus taeda* L., callus (Mott, R. L., H. A. Thomas, and G. Namkoong. 1978. Ann. Entmol. Soc. Am. 71: 564-566). The latter technique was attractive because the medium contains a minimal amount of plant secondary metabolites and it requires no anti-microbial compounds in the diet. The objectives of this research were to rear *D. frontalis* and *I. grandicollis* on tissue-cultured host pine callus and to examine the influence of host species and additions of symbiotic fungi to the rearing system for *D. frontalis.* The two fungi used were the *D. frontalis* mycangial associates *Ceratocystis minor* (Hedgecock) Hunt var. *barrasii* Taylor (Barras, S. J. and J. J. Taylor. 1973. Mycopathol. Mycol. Appl. 50: 293-305) and an unidentified basidiomycete, SJB 122 (Barras, S. J. and T. Perry. 1972. Z. Angew. Entomol. 71: 95-104) both of which are suggested to play a nutritional role for developing brood (Barras, S. J. 1973. Can. Entomol. 105: 1295-1299, Bridges, J. R. 1983. Environ. Entomol. 12: 858-861).

Experimental work was conducted during two time periods, 1978-1980 and 1986-1988. Tissue-cultured callus of loblolly pine were initiated from either 3-year-old stems or seeds while the shortleaf, *Pinus echinata* Mill., and longleaf, *P. palustris* Mill., callus were both initiated from seed. Stems were surface-sterilized, sectioned, and placed on nutrient medium at 22°C under ca. 9.29 candles/m^2 of cool white flourescent light for 4 weeks. A modified Brown and Lawrence nutrient medium (Brown, C. L. and R. H. Lawrence. 1968. For. Sci. 14: 62-64) containing 1.5 ml of the recommended vitamin mixture, 0.345 ml kinetin, 15 ml each of nitrate, sulfate,

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halide, PbMo, and FeEDTA solutions, 6.75 ml 2,4-dichlorophenoxyacetic acid, and 30 g of sucrose was used throughout the study. Agar concentration was varied from 1 to 3% in the 1.5 L solution and pH was adjusted to 5.5 in all trials. Seeds were scarified and maintained in moist aseptic peroxide conditions for 4 days (2 days in a 1% peroxide solution followed by 2 days in a 0.03% peroxide solution) at 22° C after which the meristematic tissue was removed, finely chopped, and placed on nutrient agar at 22°C under ca. 9.29 candles/m^2 of cool white flourescent light for 4 weeks. The callus tissue resulting from either technique was transferred to nutrient agar medium. Callus tissue was subdivided and plated on fresh nutrient medium bi-weekly.

Beetle eggs were collected from naturally infested loblolly and shortleaf pine in North Carolina. Eggs of *I grandicollis* were only used during the initial study period while *D. frontalis* eggs were used during both periods. Fungal cultures were maintained on malt extract agar and added to the callus media as: 1) live mycelia, 2) fungal filltrates which were centrifuged for 10 minutes at 10,000 rpm and twice-filtered using an aspirator and 45 μm milipore filter, or 3) fungal extracts which were centrifuged, filtered and homogonized in a Wearing blender then re-centrifuged and twice-filtered prior to placement on the callus.

Eggs were surface sterilized in a Zephiran: H_2O solution (1:750), rinsed with sterile H_2O and placed in niches cut into the callus tissue. Niches were excavated using a dissecting probe to make a small (1-2 mm deep) hole in the callus tissue. Removed tissue was then securely replaced over the eggs to simulate natural egg placement in niches. All *I. grandicollis* eggs (n=200) were placed on loblolly callus growing on nutrient agar with β-sitosterol (60 mg/liter) added as a sterol source. The *D. frontalis* eggs were placed on the following callus treatments: 180 on loblolly callus with β-sitosterol, 120 on shortleaf callus with β-sitosterol, 120 on long leaf callus with β-sitosterol, 24 on loblolly callus with live SJB-122, 24 on shortleaf callus with live *C. minor* var. *barrasii,* and 30 each (120 total) on loblolly callus with SJB-122 or *C. minor* var. *barrasii* filtrate or extract with β-sitosterol. A total of 588 *D. frontalis* eggs were used. All treatments were monitored for 30 to 60 days after egg placement and then dissected to determine life stage of the insect at time of death.

While egg hatch exceeded 90%, no egg to adult survival was observed for *D. frontalis* (Table 1). Indeed, no larvae survived to pupation and most died without completing a single molt. Thirteen *I. grandicollis* survived to become adults (Table 1) and there was also evidence that more of the population survived beyond the first instar. Indeed, ca. 50% of the *I. grandicollis* larvae fed through the callus and died while tunnelling in the agar.

The callus tissue often became very moist soon after egg placement and the only attempt at controlling callus moisture content was to vary ager concentration from 1 to 3% in the nutrient media. Initial agar concentration was 1% and modified when callus tissue was subdivided and transferred to fresh media prior to egg placement. The higher agar concentrations did appear to result in less moist callus tissue. Excessive moisture has previously been described as a critical factor in rearing bark beetles (Bedard, W. D. 1966. Ann. Entomol. Soc. Am. 59: 931-938, Schmidt, F. H. 1966. Can. Entomol. 98: 1050-1055) and may have contributed to our lack of success. Also, given the apparent inability to molt by larval *D. frontalis,* there also may have been a nutritional deficit in the callus tissue. The sterol source provided was placed on the nutrient media and may not have been

Table 1. Egg to adult survival (%) of two bark beetle species on host pine callus with (+) or without (-) an added sterol (β-sitosterol) and/or the *D. frontalis* mycangial fungi (SJB-122=unidentified basidiomycete or *Cmb=Ceratocystis minor* var. *barrasii;* whole=live mycelia, filtrate=centrifuged, twice filtered mycelial suspension, or extract=twice filtered, centrifuged homogenate of the mycelial suspension).

		Treatment			
Insect	Callus	No. of Eggs	Sterol	Fungus	% Survival
D. frontalis	loblolly	180	+	none	0
		24	-	SJB-122, whole	0
		30	+	filtrate	0
		30	+	extract	0
		30	+	*Cmb*,filtrate	0
		30	+	extract	0
	shortleaf	120	+	none	0
		24	-	*Cmb*,whole	0
	longleaf	120	+	none	0
I. grandicollis	loblolly	200	+	none	6.5

incorporated into the callus tissue although Mott et al. (1978) successfully reared *D. frontalis* using the same technique. Some *I. grandicollis* did survive to adulthood and this may be the result of differences in suitability of different host materials for the two species. In the field, *D. frontalis* exhibits a more restricted host suitability range, generally attacking live standing pines while *I. grandicollis* utilizes host material ranging from live standing trees to downed timber and slash (Thatcher, R. C. 1960. U.S. Dept. Agric. For. Serv. Occas. Pap. 180). Also, the *I. grandicollis* wandered more while feeding and may thus have been exposed to more sterol than the *D. frontalis* larvae. Since no *D. frontalis* larvae in any treatments survived, this work suggests that under the given test conditions that these fungi did not play a nutritional role in the development of this beetle.

The initial culturing and continued maintenance of callus tissue is a time consuming process requiring strict rearing conditions. There can also be a problem with microbial contamination because no antimicrobial compounds are used in the tissue. Given these difficulties and our inability to duplicate the success of Mott et al. (1978) using this technique, the yeast-fortified ground phloem diets may be easier to use for aseptically rearing beetles. However, the use of antimicrobial compounds in these phloem diets prevents these techniques from being a useful tool to examine bark beetle-fungal associations.

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